

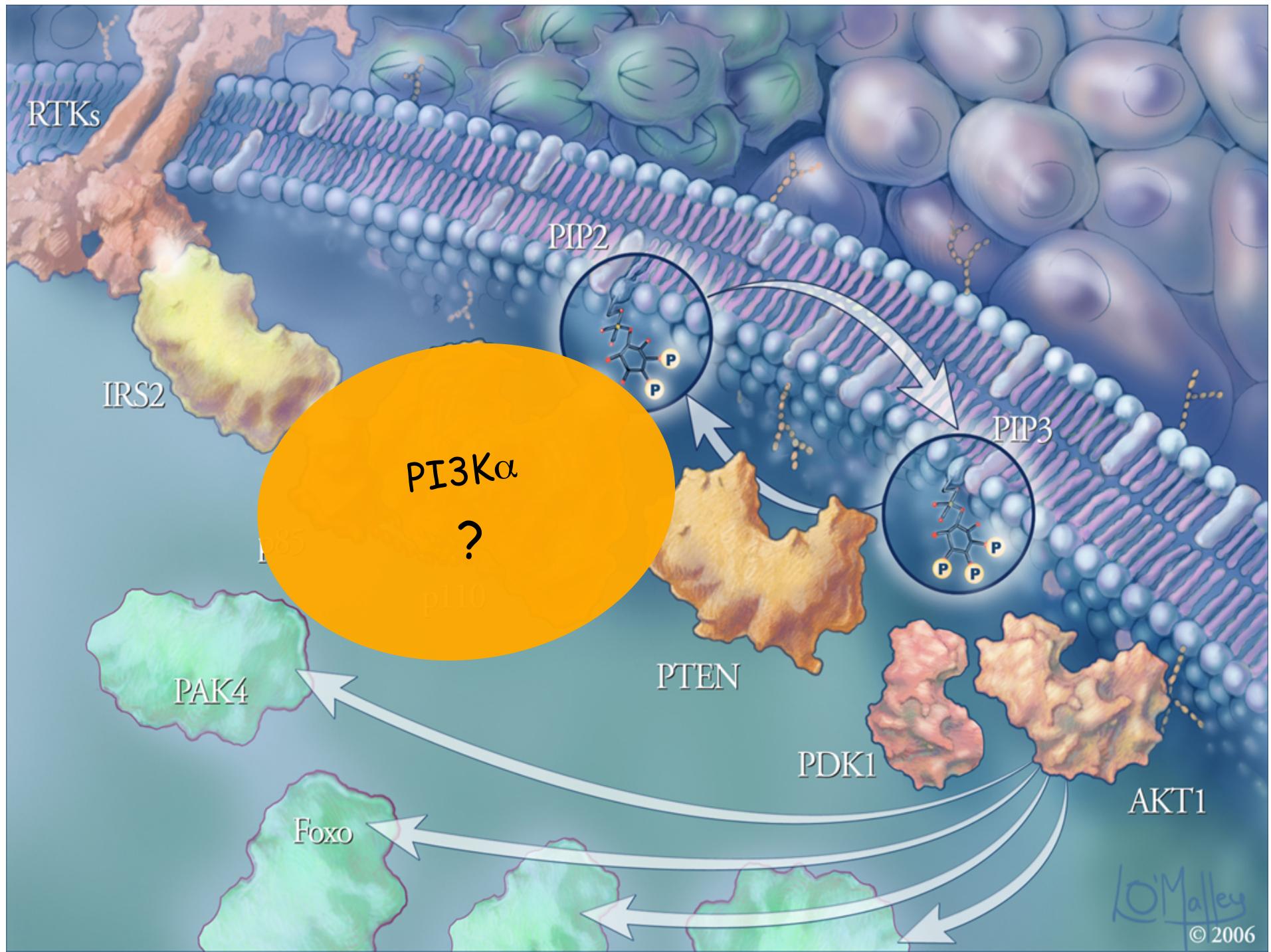
PI3K in cancer: structural basis for the mechanism of activation

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Johns Hopkins University

February 14, 2011

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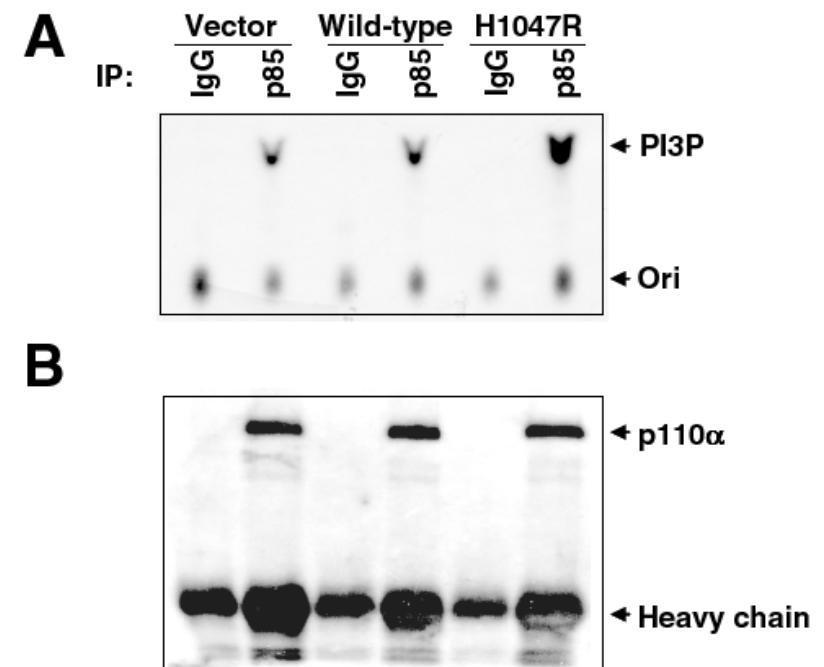


High Frequency of Mutations of the PIK3Ca Gene in Human Cancers

1047R Mutation gain of function



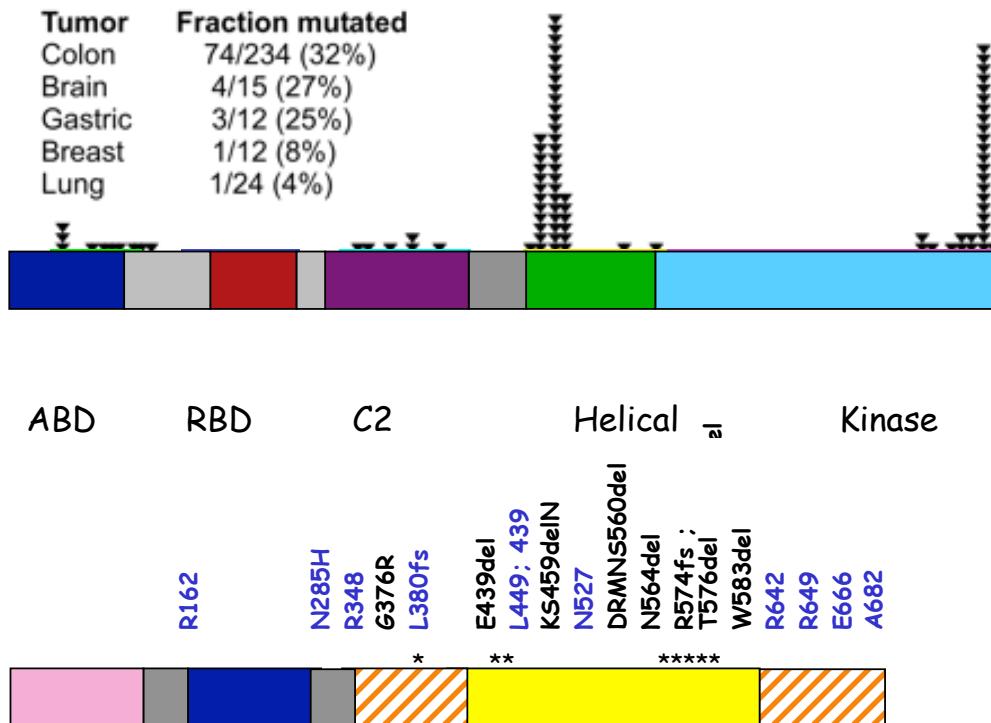
ABD RBD C2 Helical Kinase



Samuels, et al, Science 2004

Bader, et al, Nat. Rev. Cancer 2005

High Frequency of Mutations of the PIK3R1 Gene in Human Cancers



The Cancer Genome Atlas Research, Nature 2008

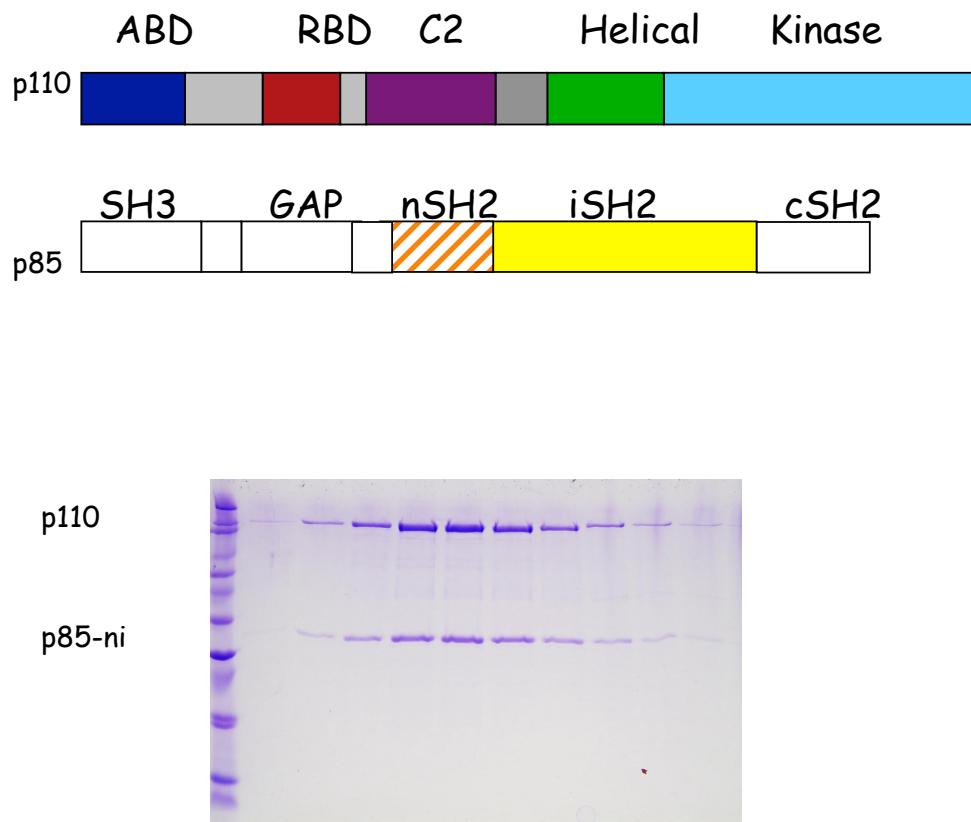
Jaiswal, et al, Cancer cell 2009

"Hot-spot" mutations in cancer

- Gain-of-function point mutations in oncogenes seen recurrently in human tumors.
 - BRAF V600E inhibits apoptosis and promotes invasiveness
 - K-RAS G12C, G12D, G12V pancreatic cancer.
 - EGFR L858R in lung cancer -sensitivity to erlotinib, T790M- resistance to erlotinib.

What is the mechanism of activation of the oncogenic mutations?

Structure of p110 α /niSH2 heterodimer

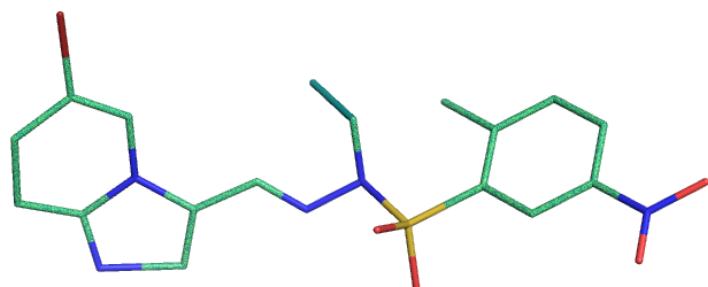
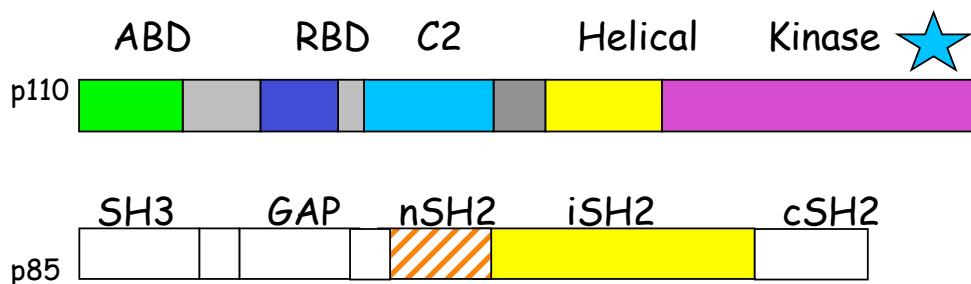


Yu, et al, 1998

Huang, et al, 318 Science 2007

Space group	P2 ₁ 2 ₁ 2 ₁
Cell dimensions a, b, c (Å)	115.1, 117.1, 151.6
Resolution (Å)	3.05
Measured Reflections	270,209
R_{sym}	7.2 (43.3)
$\langle I / \sigma I \rangle$	9.4 (2.1)
Completeness (%)	98.7 (88.2)
Refinement	
Resolution (Å)	46.4-3.05
$R_{\text{work}} / R_{\text{free}}$	26.3 / 32.3
No. residues	1,133
No. atoms Protein	9,365
Average B -factor	85.7
R.m.s. deviations	
Bond lengths (Å)	0.01
Bond angles (°)	1.5

Structure of H1047Rp110 α /niSH2 heterodimer



Imidazopyridine based inhibitors PIK-75

Crystal	H1047R
Space group	p110 α /p85 α +wot $P2_12_12_1$
Cell dimensions	$a = 115.3 \text{ \AA}$ $b = 121.4 \text{ \AA}$ $c = 152.5 \text{ \AA}$

Data Collection Statistics

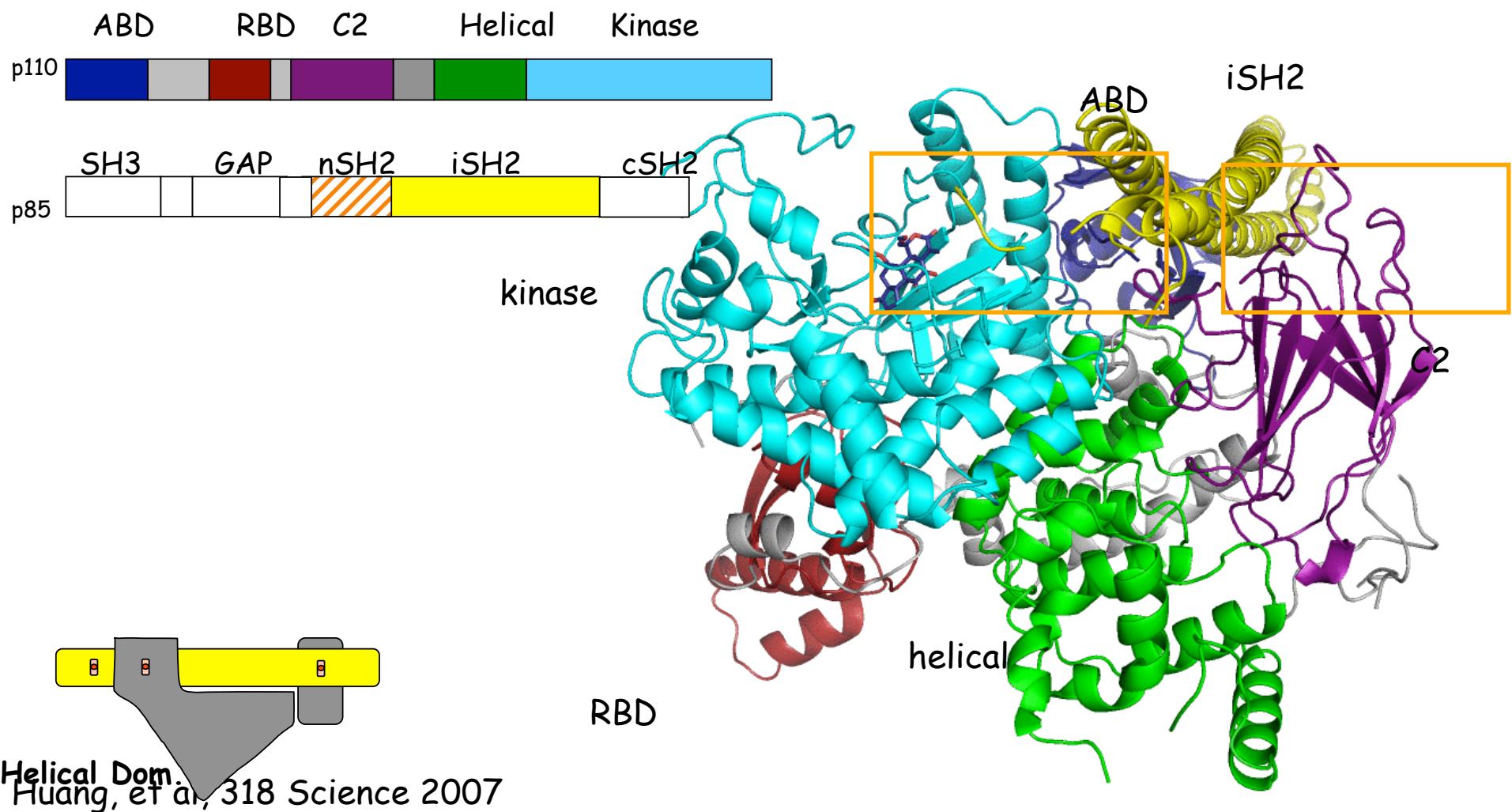
X-ray Source	APS
Wavelength (\AA)	0.97929 \AA
Resolution(\AA)	50-2.8 (2.9-2.8)
Measured Reflect.	362,120
Unique Reflections	53,371
I/σ	23.6 (2.0)
Completeness (%)	99.8 (99.6)
$R_{\text{merge}} (\%)$	9.3(68.9)

Refinement

$R_{\text{cryst}} (\%)$	24.4
R_{free}	30.9
Bfactor (protein) \AA^2	75.87

Mandelker, et al PNAS (2009) 16996-7001

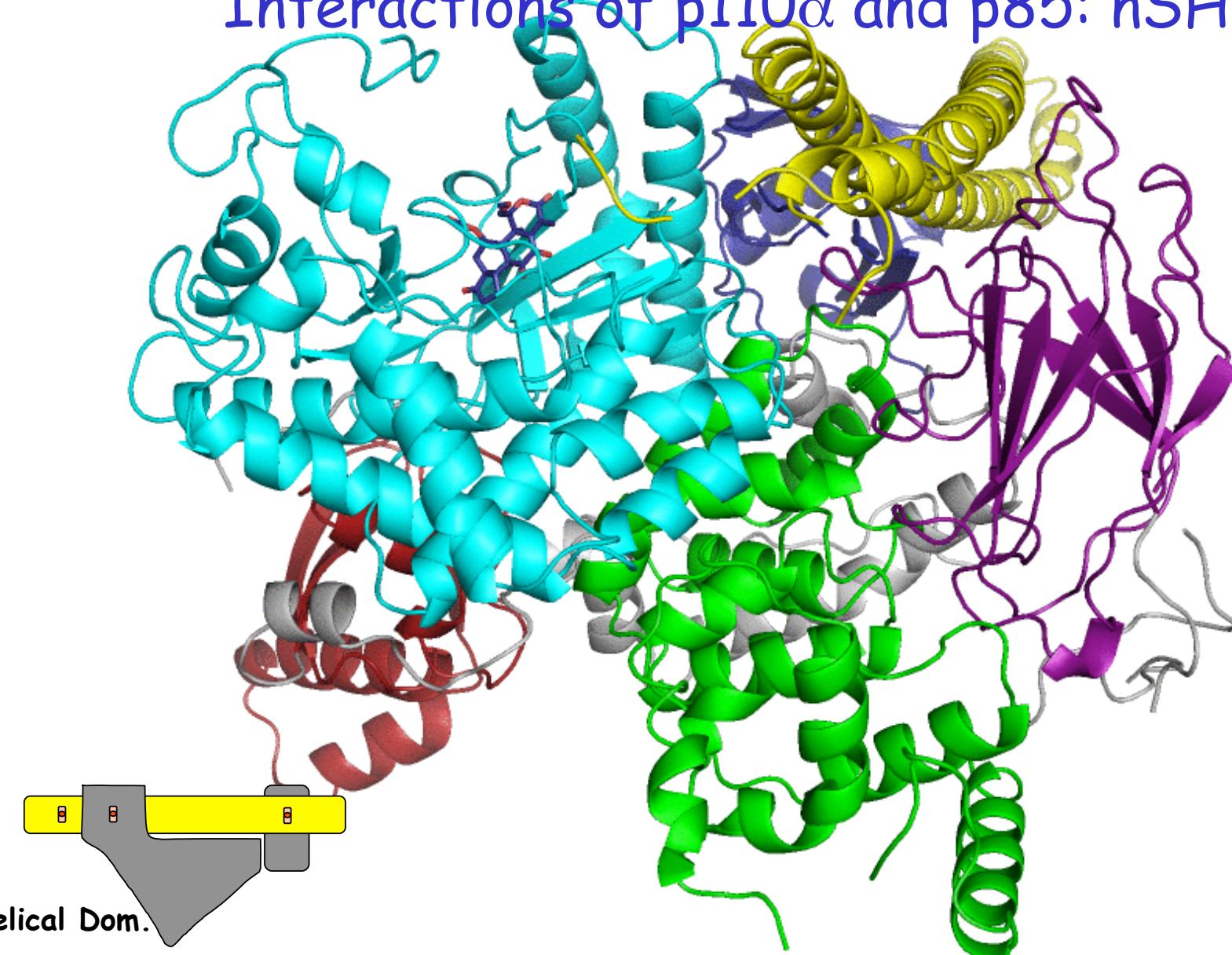
Structure of p110 α /niSH2 heterodimer



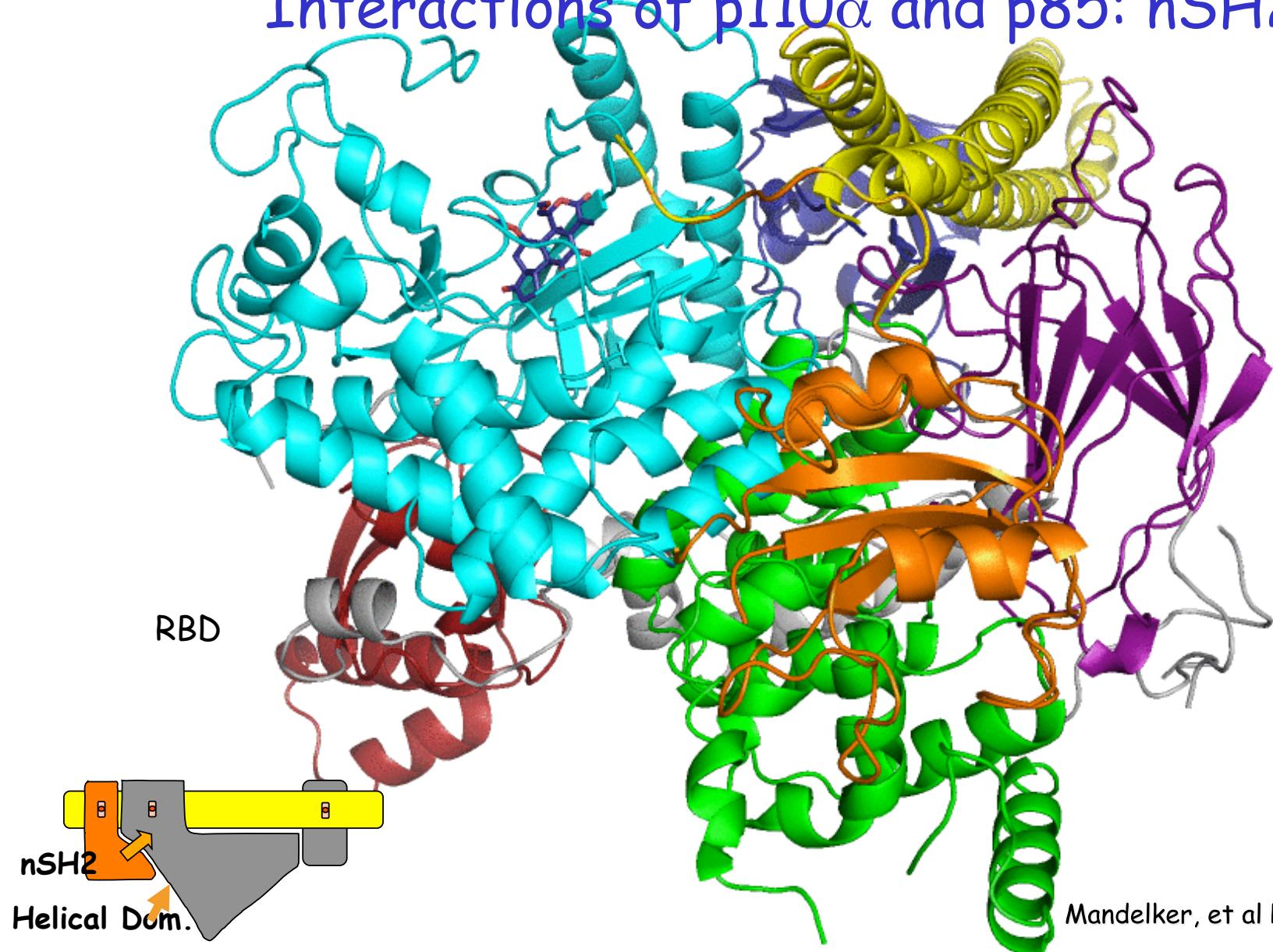
Interactions of p110 α and p85: nSH2

nSH2 acts as a scaffold for
three domains of p110 α

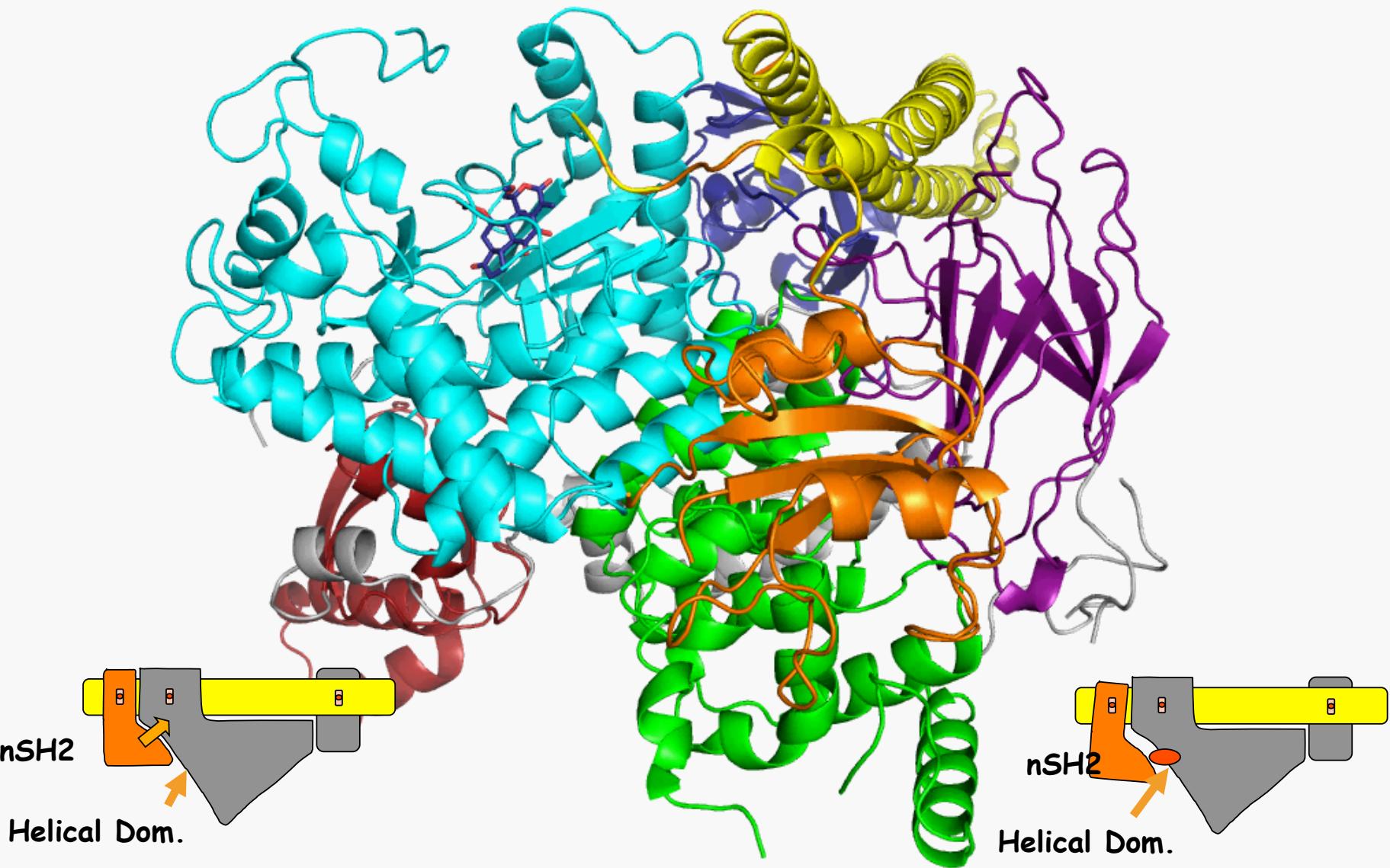
Interactions of p110 α and p85: nSH2



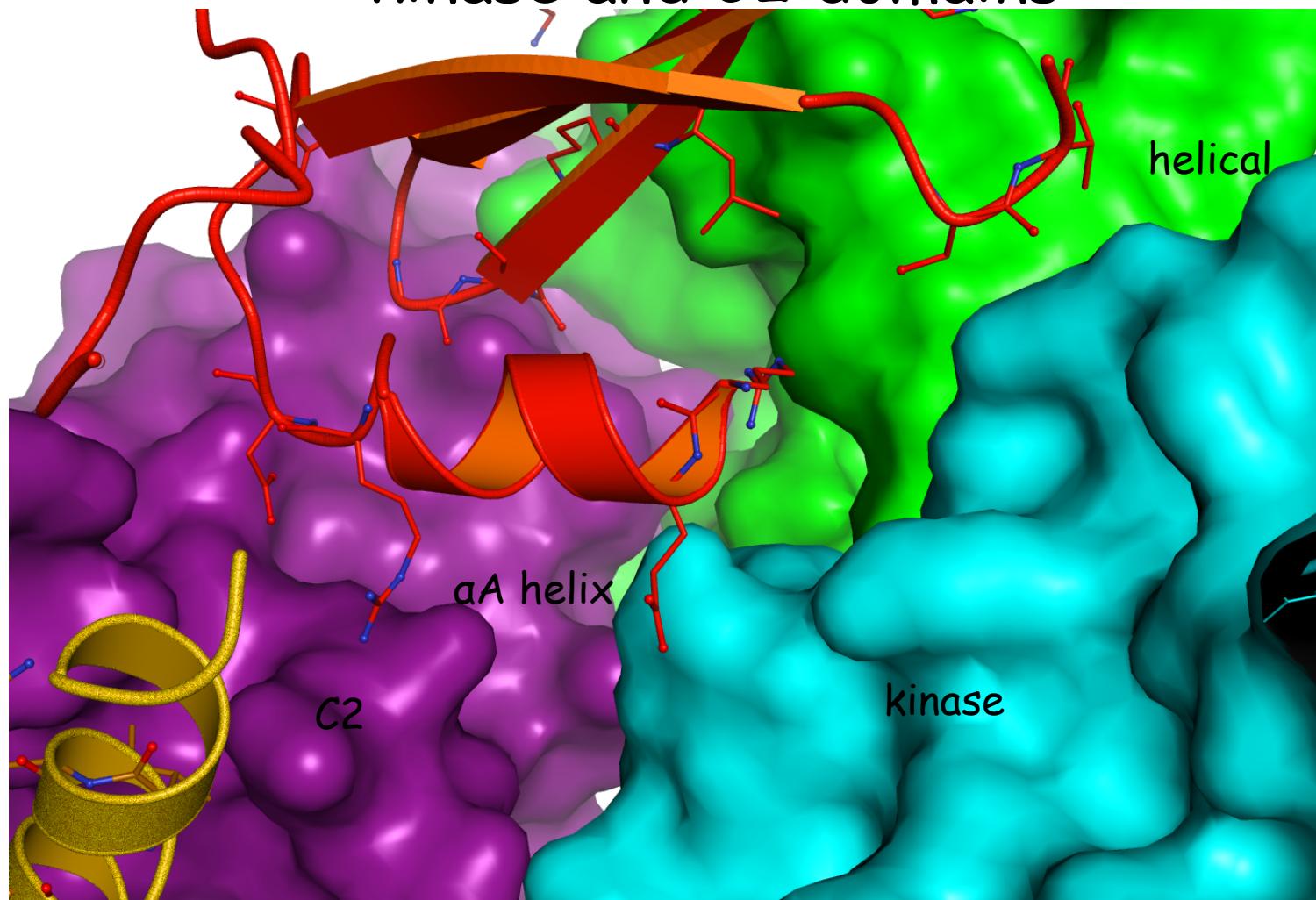
Interactions of p110 α and p85: nSH2



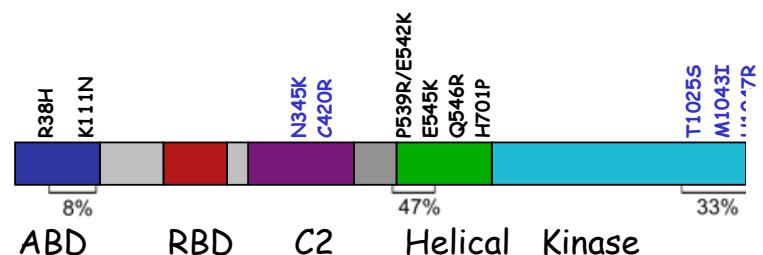
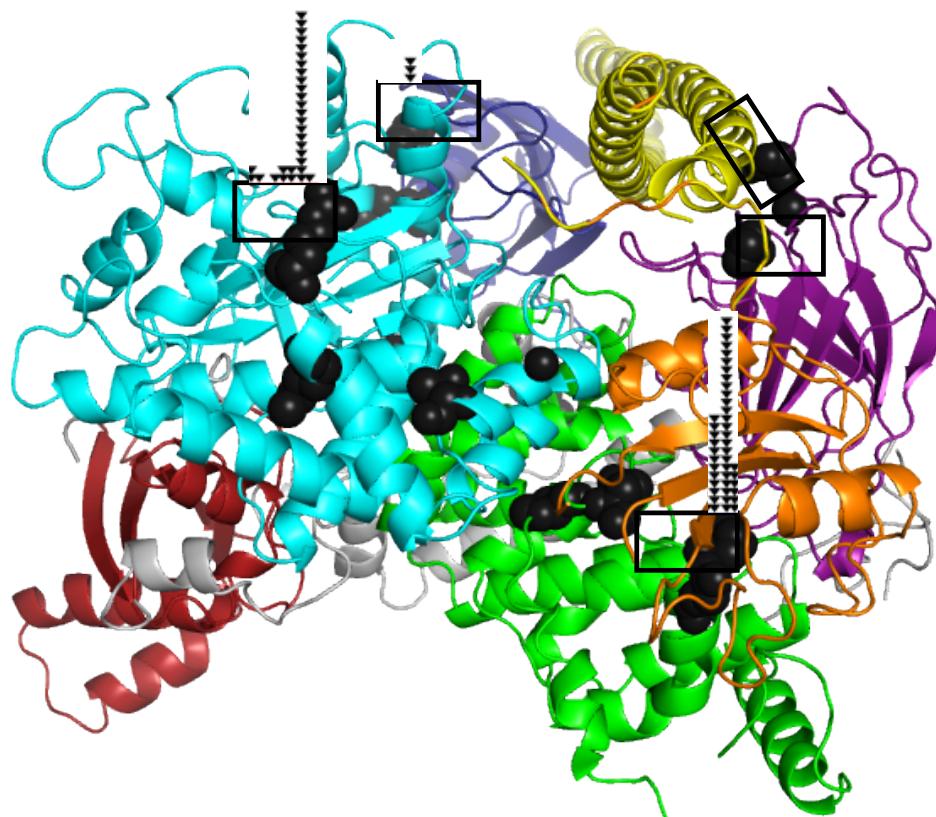
Mandelker, et al PNAS (2009) 16996-7001



α A helix of nSH2 fits into cavity between kinase and C2 domains



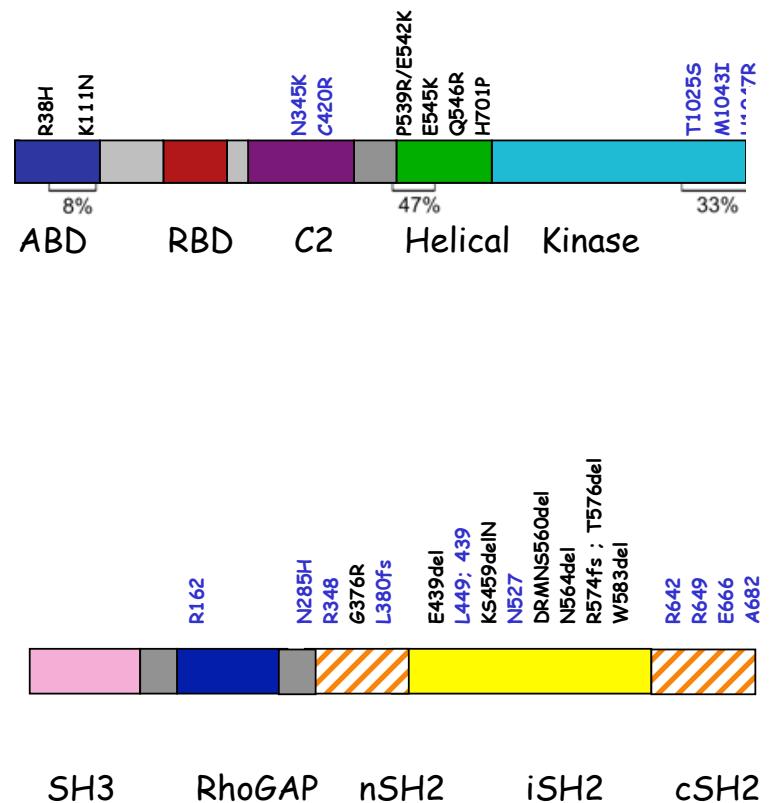
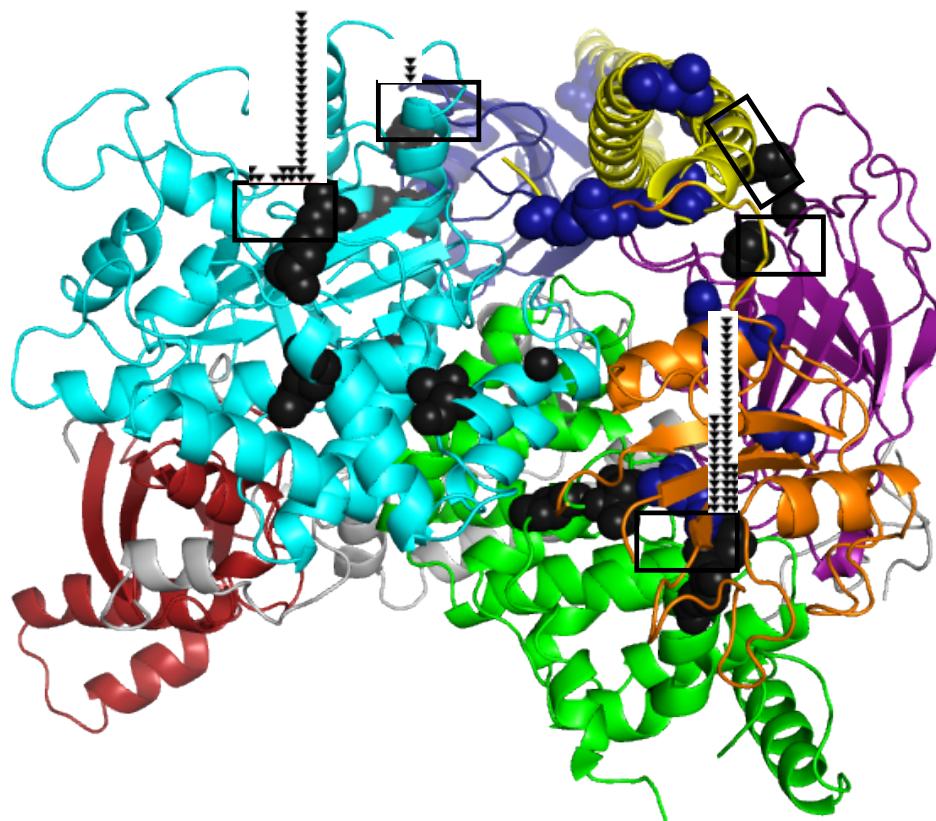
Cancer-Specific Mutations



Number of tumors with the indicated mutation						
Somatic mutations	Breast	Colon	Others	Total	Percent of p110 mutations	
p85						
R38H				2	2	0.6
K111N	2	2		2	0.6	
Linker						
N345K♦	2	1		3	0.9	
C420R♦	7	2		9	2.7	
P539R♦	3	1		4	1.2	
E542K♦	20	12	8	40	12	
E545K♦	33	26	24	83	24.9	
E545A			12	12	3.6	
E545G	2		1	3	0.9	
Q546K♦		6	2	8	2.4	
Q546P		1	1	2	0.6	
H701P	2		1	3	0.9	
T1025S	1			1	0.3	
M1043I		2	1	3	0.9	
M1043V	1			1	0.3	
H1047L	11	3	2	16	4.8	
H1047Y	1	2	6	9	2.7	
H1047R♦	81	20	32	133	40	

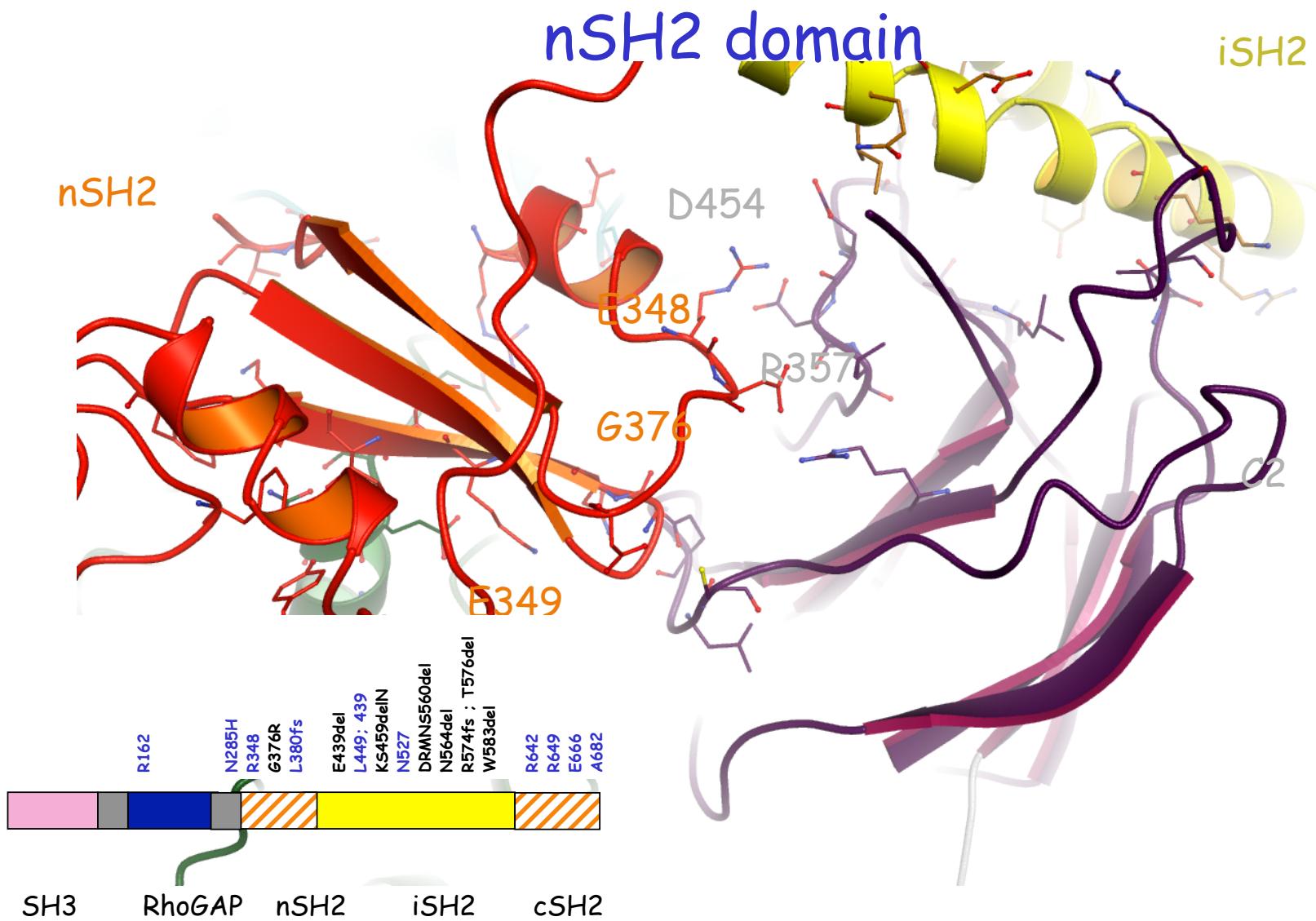
Gymnopoulos, et al PNAS 2007

Cancer-Specific Mutations



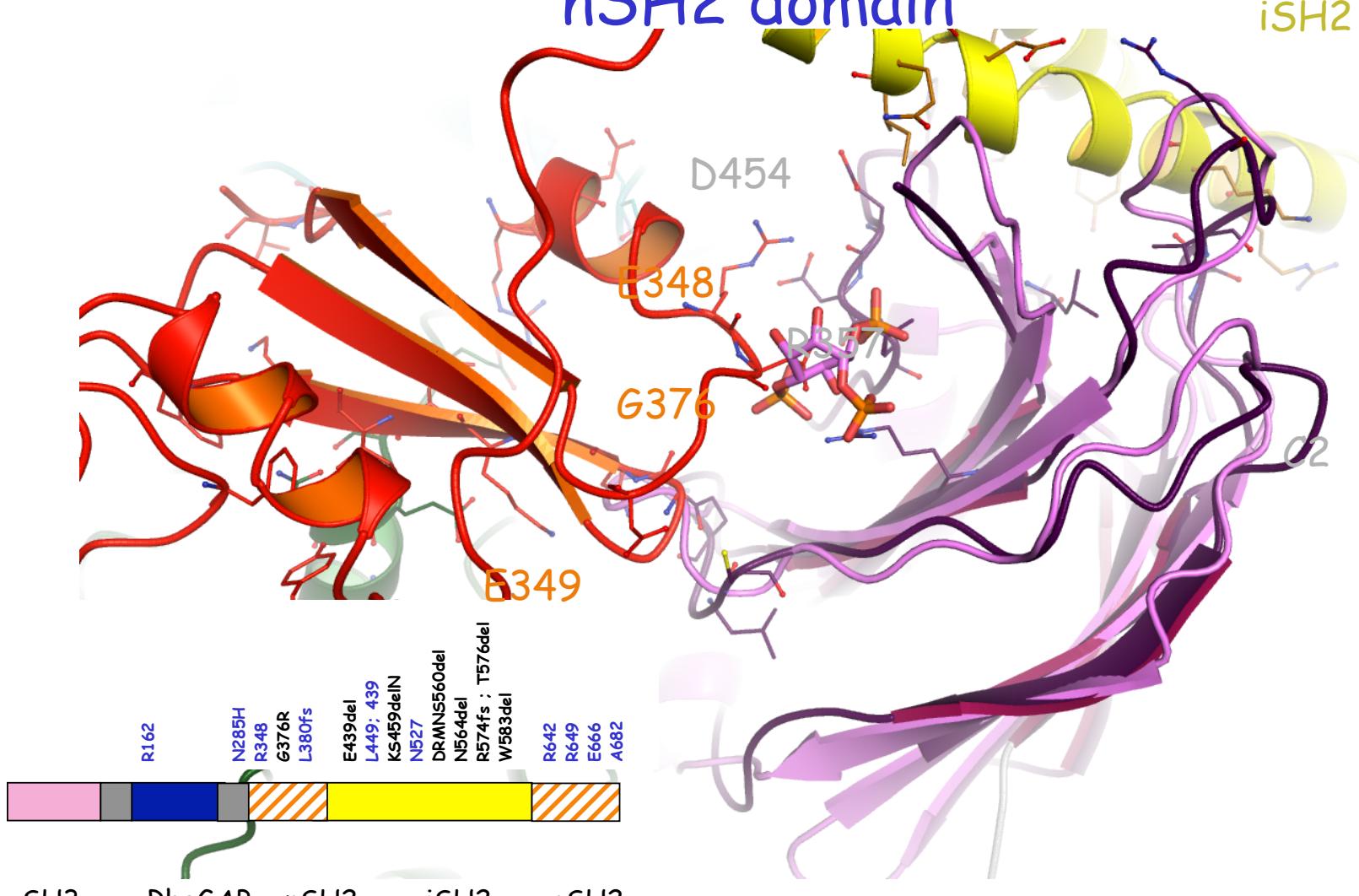
TCGRN, Nature 2008.
Jaiswall, Cancer Cell 2009

Glioblastoma Specific Mutations:



Glioblastoma Specific Mutations:

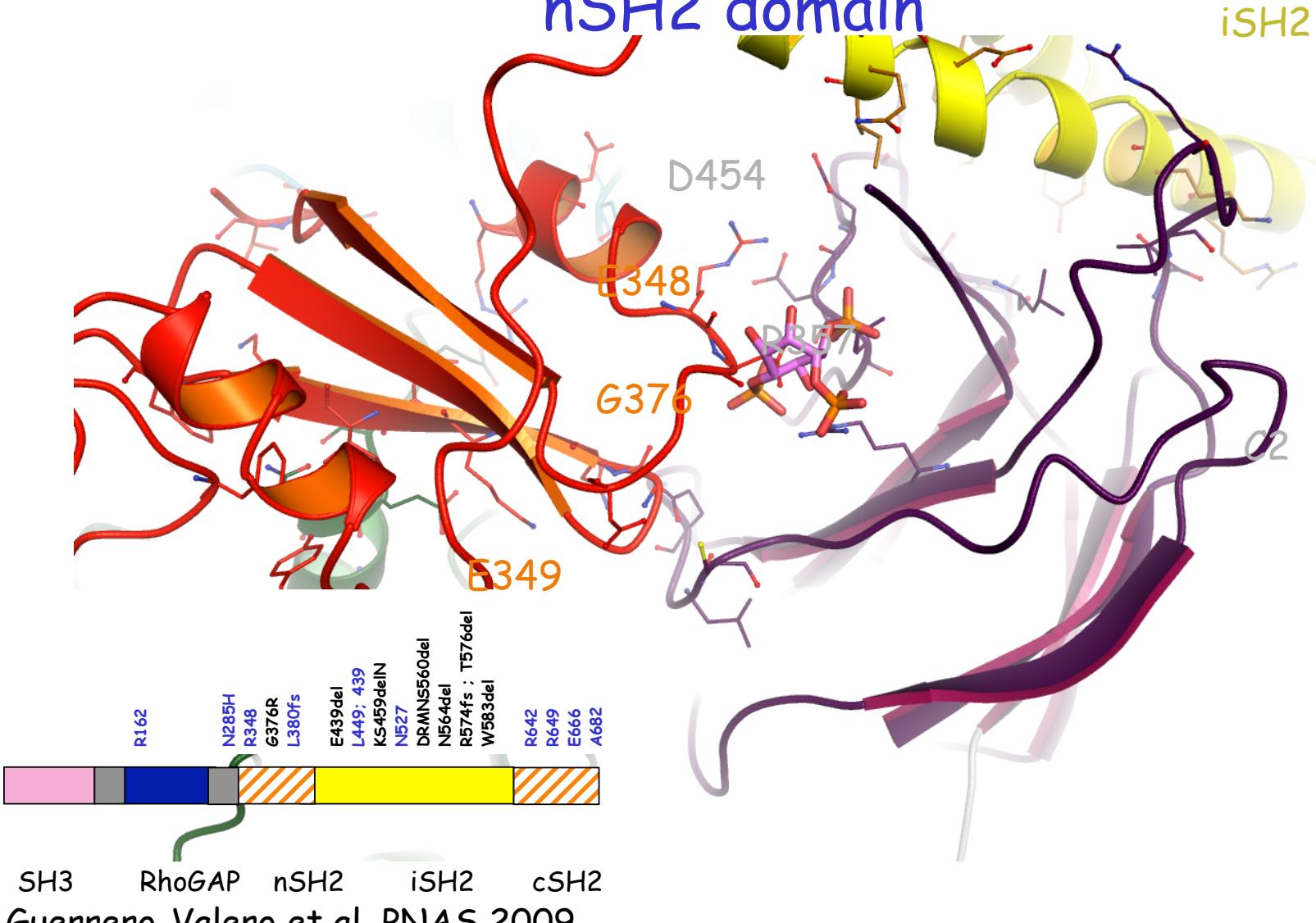
nSH2 domain



Guerrero-Valero et al, PNAS 2009

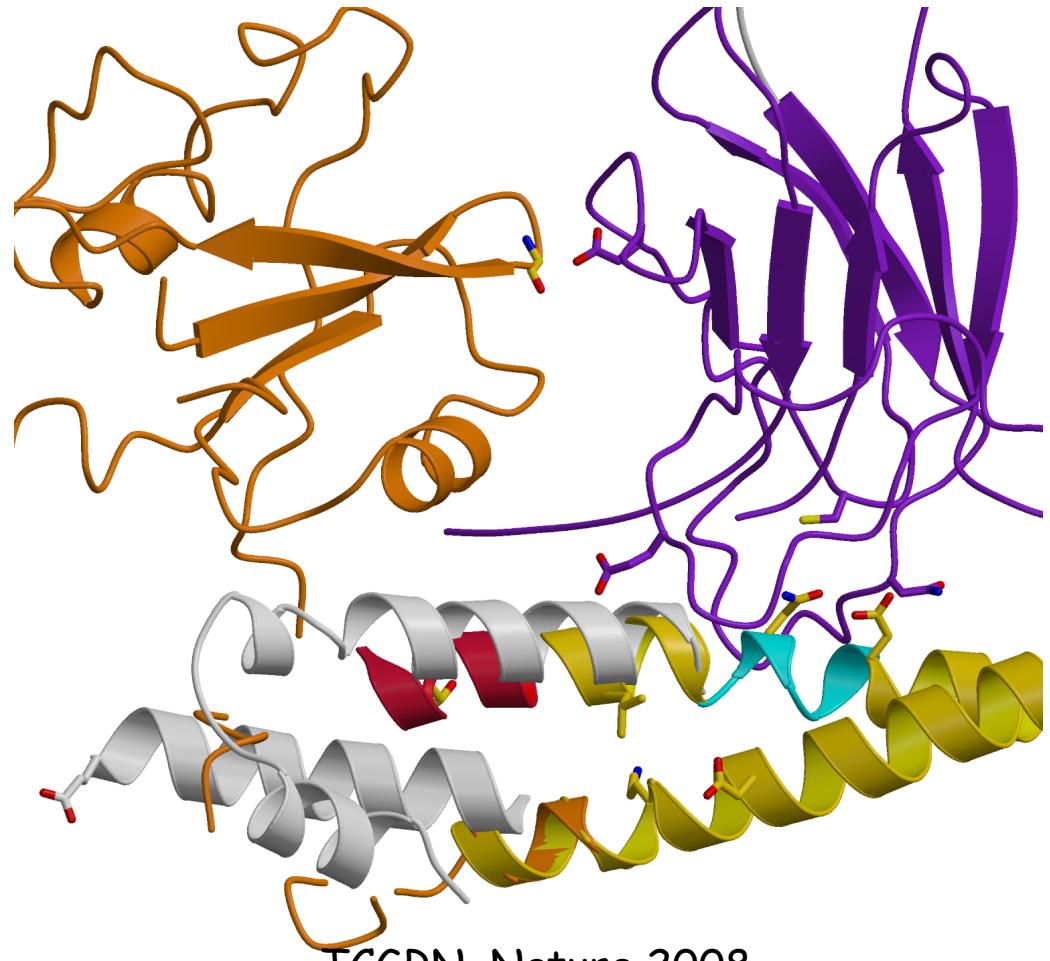
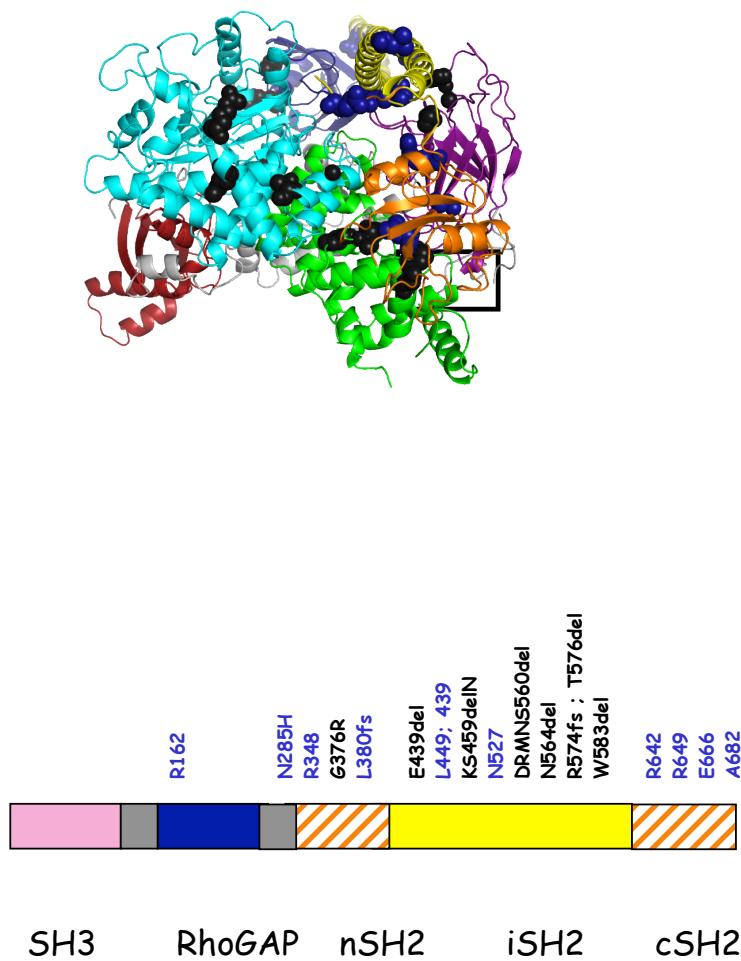
Glioblastoma Specific Mutations:

nSH2 domain

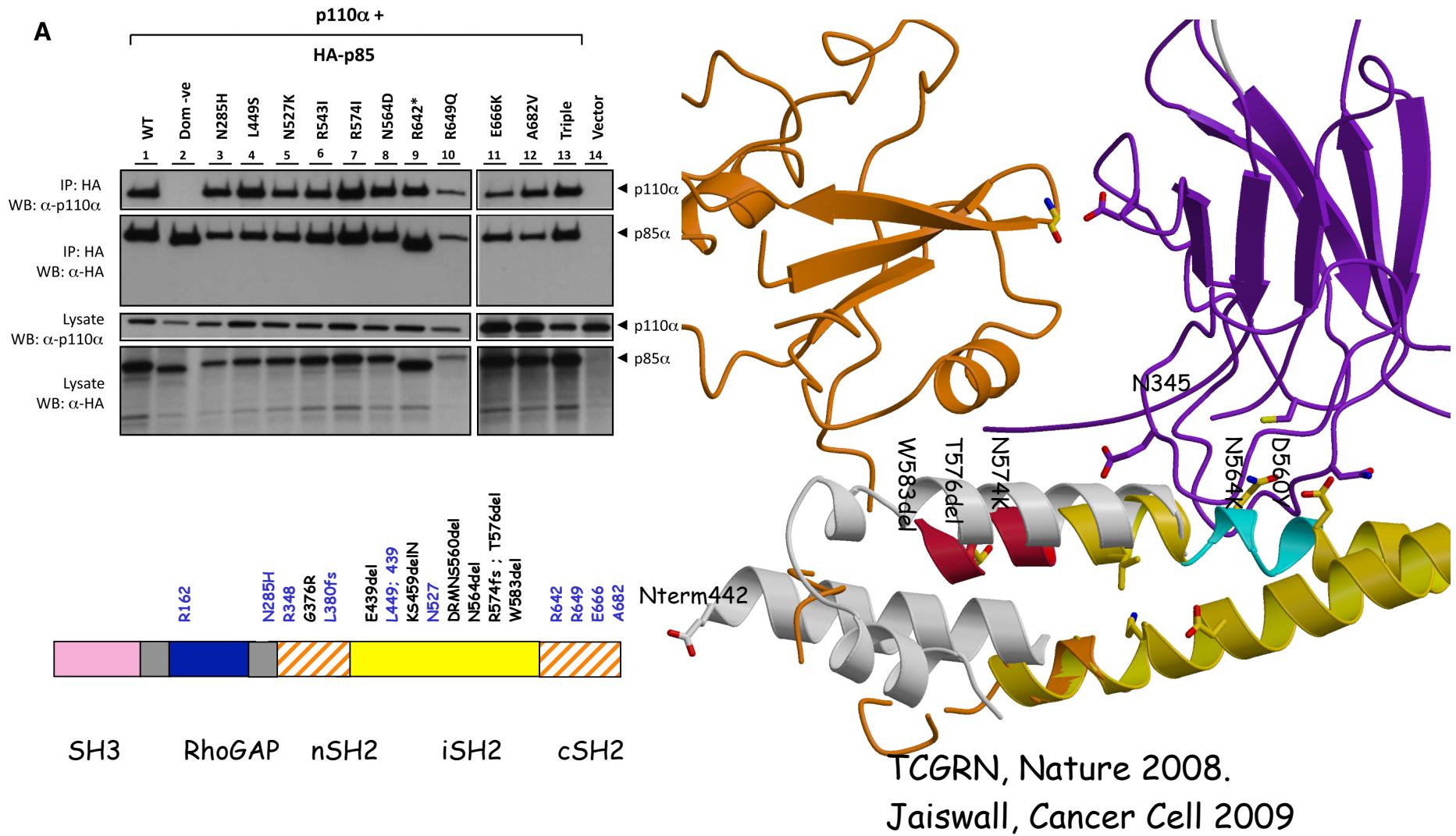


Guerrero-Valero et al, PNAS 2009

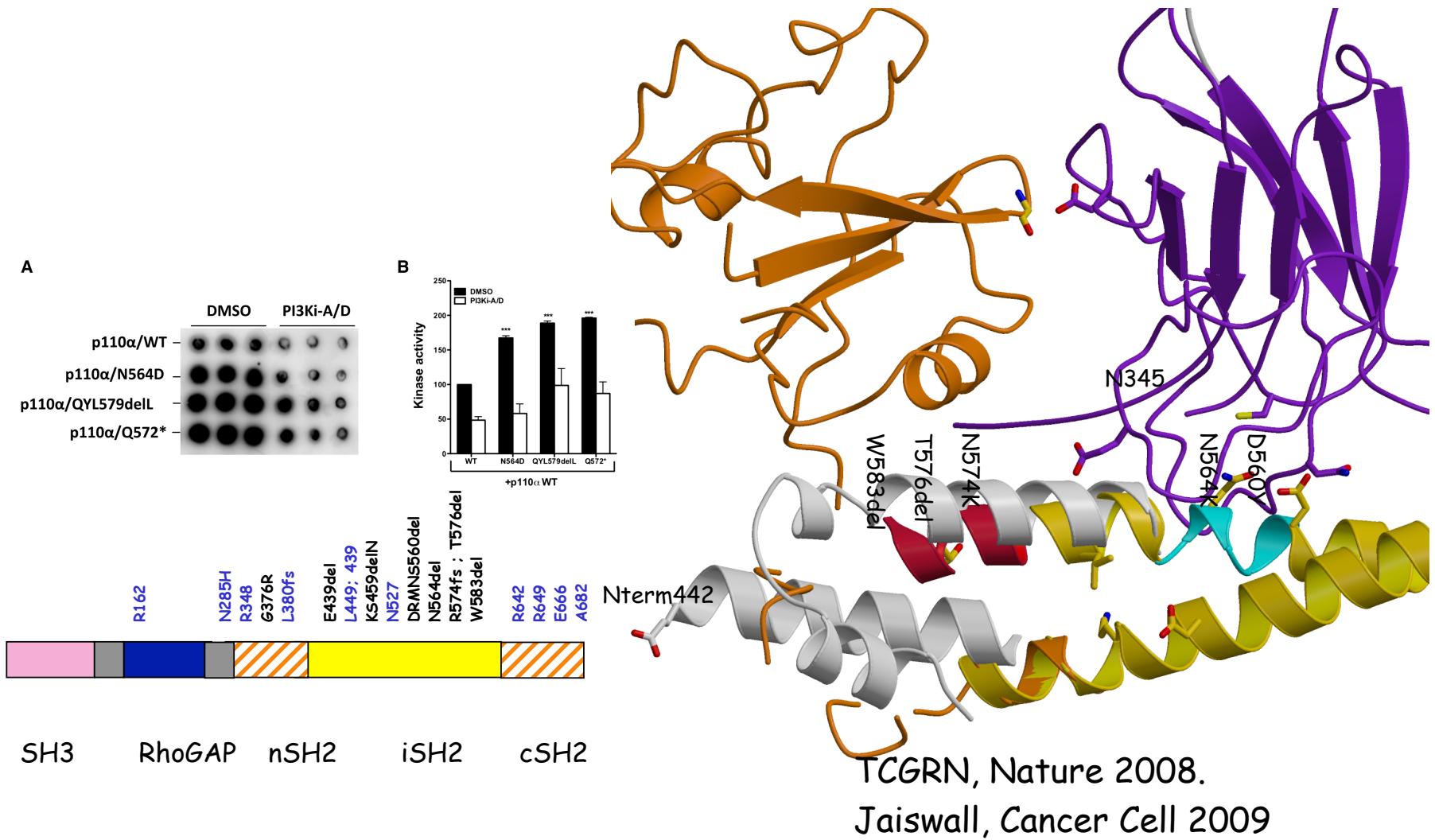
Glioblastoma Specific Mutations: iSH2 domain



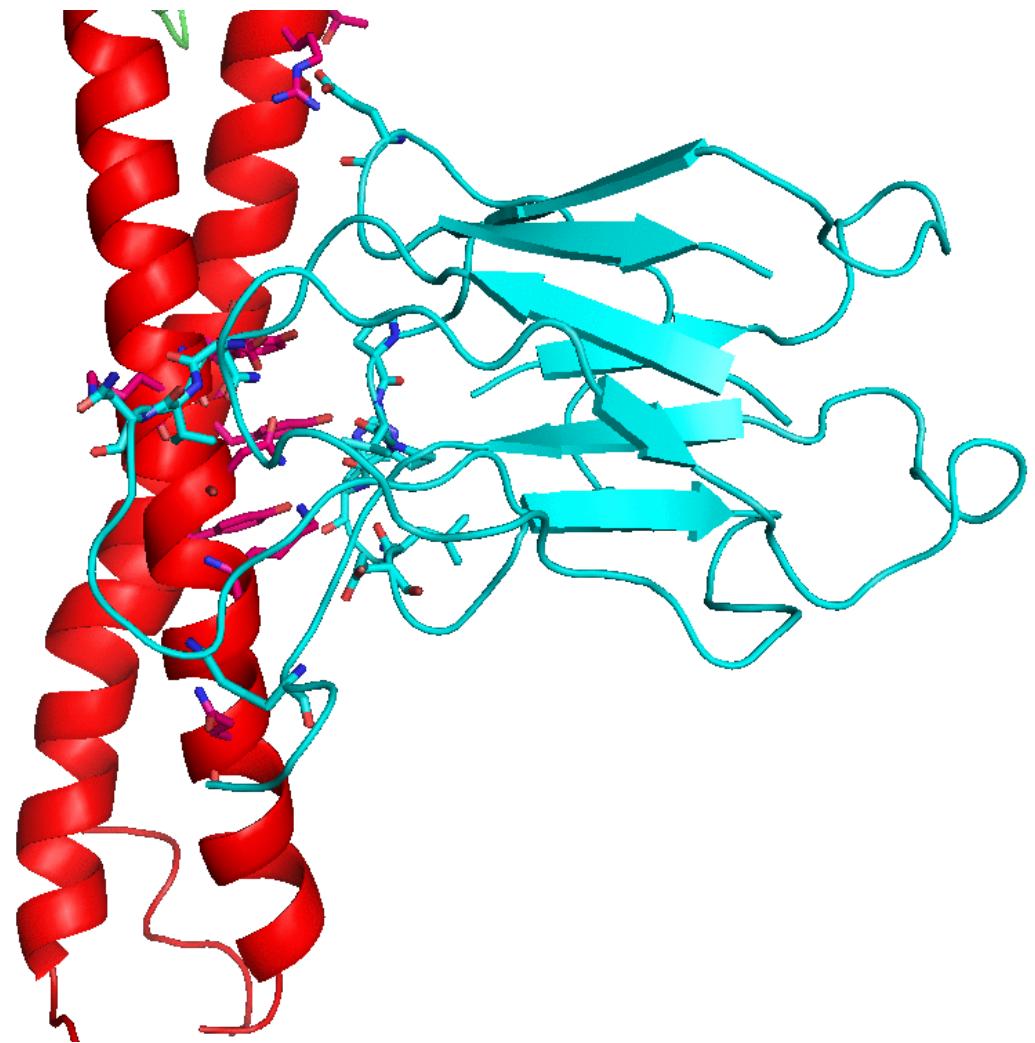
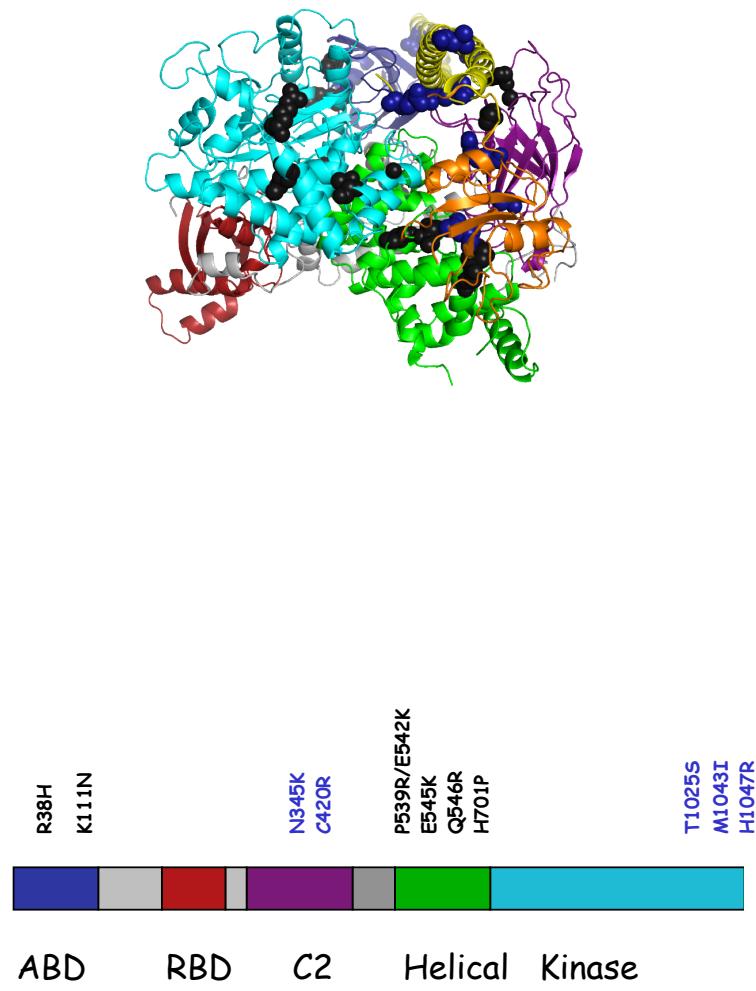
Glioblastoma Specific Mutations: iSH2 domain



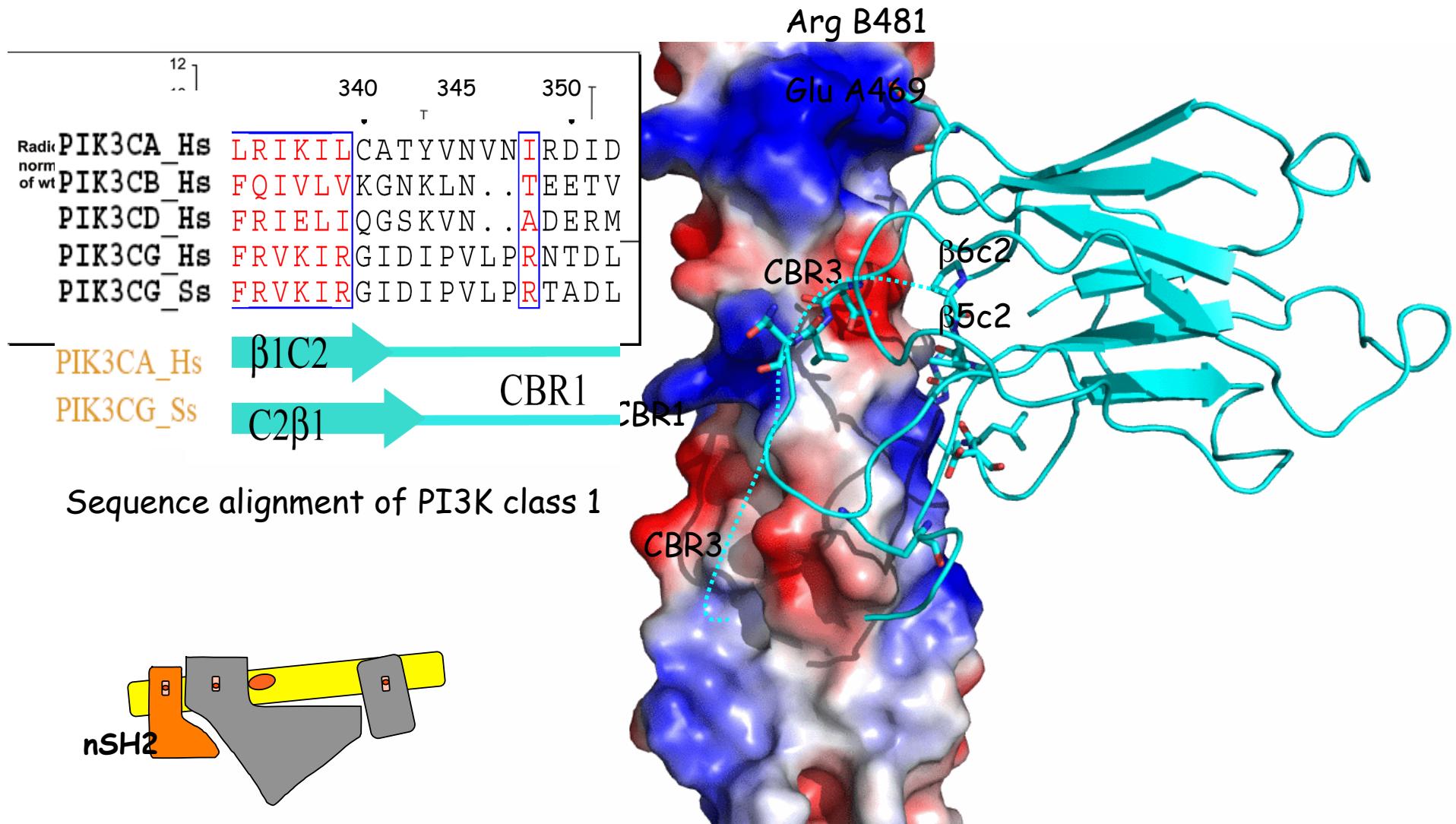
Glioblastoma Specific Mutations: iSH2 domain



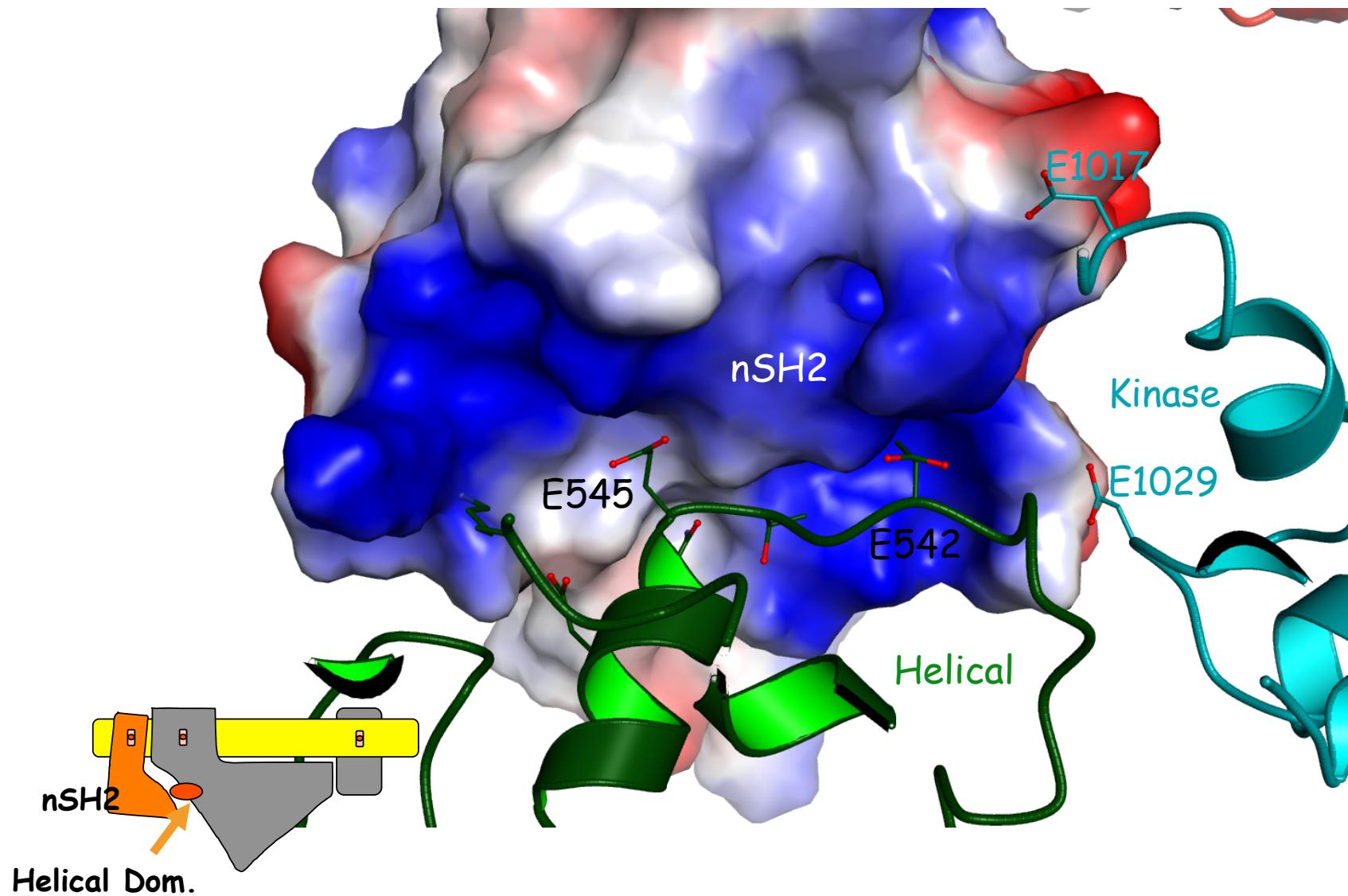
Cancer Specific Mutations: C2 Domain



Interactions of p110 α and iSH2

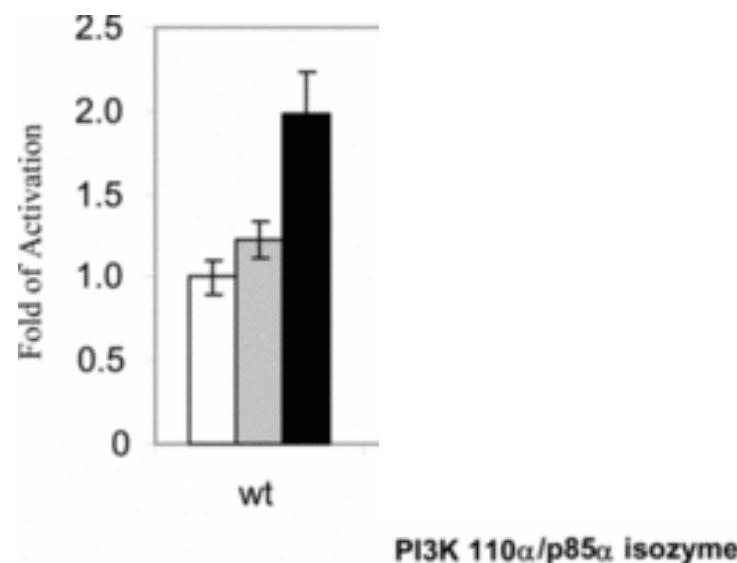


Interactions of p110 α and p85: nSH2-helical



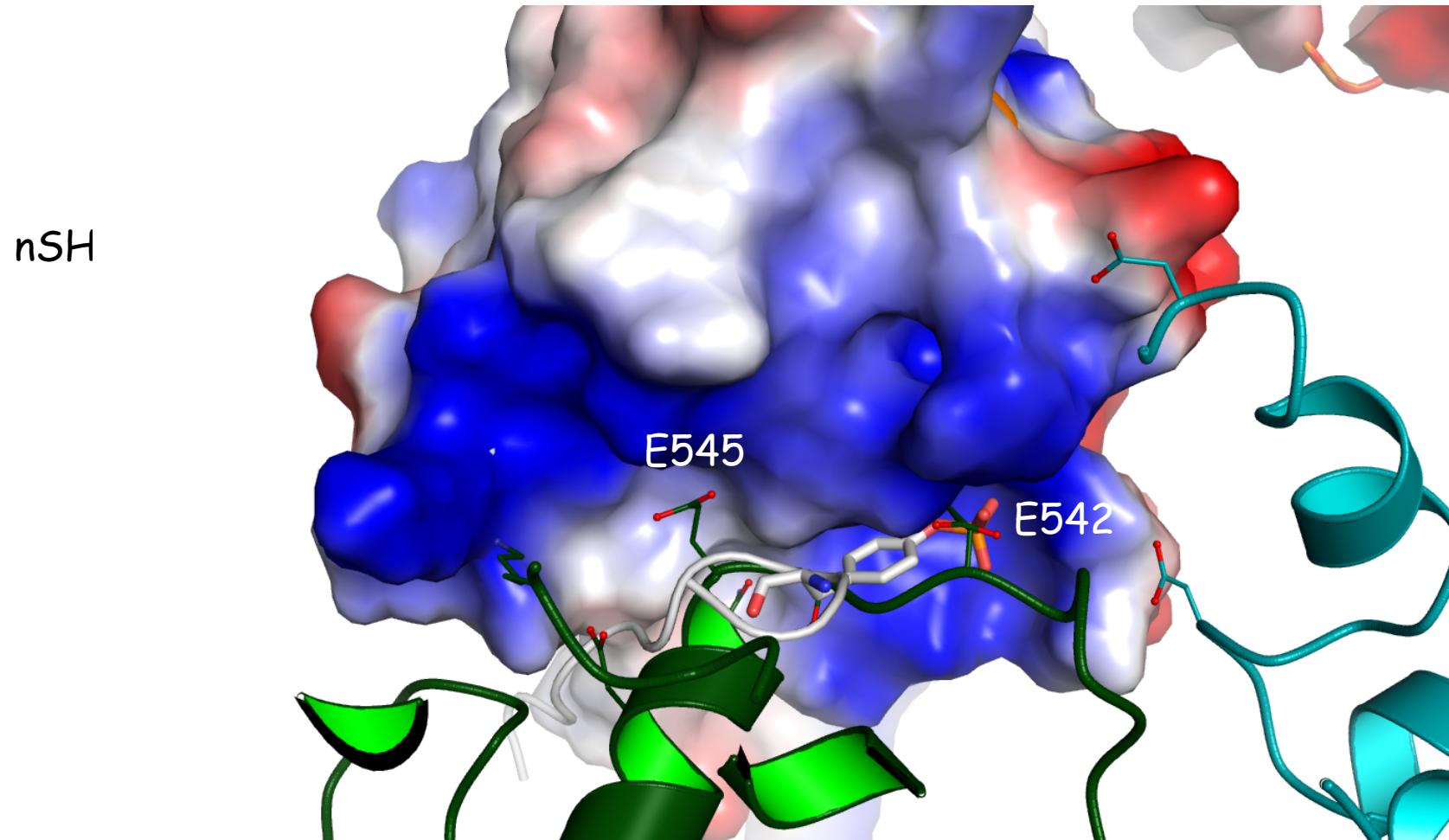
Mechanism for releasing nSH2-mediated inhibition of p110 α

- Phospho-peptide (of IRS-1, PDGFR, etc.) binding to PI3Ka increases enzyme activity of WT



wt PI3Ka lipid kinase activity in the presence of 0 nM (white bars), 20 nM (grey bars) and 200 nM (black bars) phosphorylated IRS-1 protein

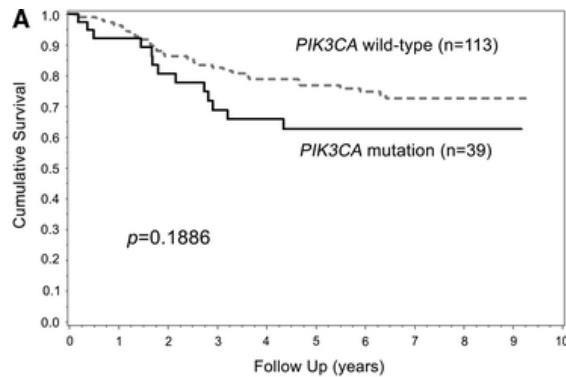
Phospho-peptide binds between nSH2 and helical domain, displacing nSH2



Nolte et al. *Nat Struct Biol.* (1996)

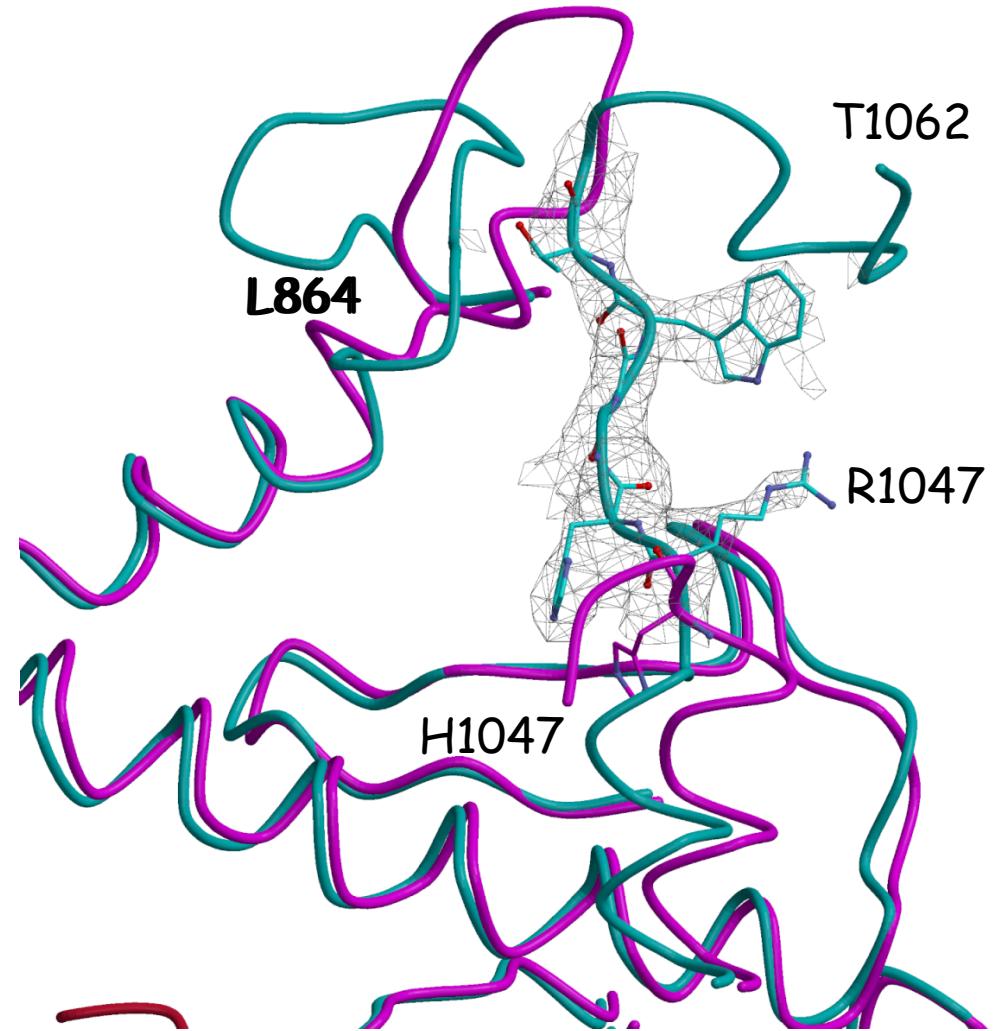
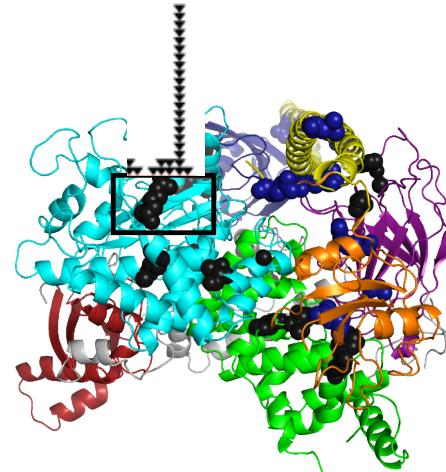
Cancer Specific Mutations: Kinase Domain

- The most frequent oncogenic mutation in PIK3CA is located in kinase domain of p110 α .
- Breast and uterine cancer patients harboring this mutation have been found to have a poorer prognosis than those with other mutations in PIK3CA.



Lai YL. et al *Ann Surg Oncol.* (2008)

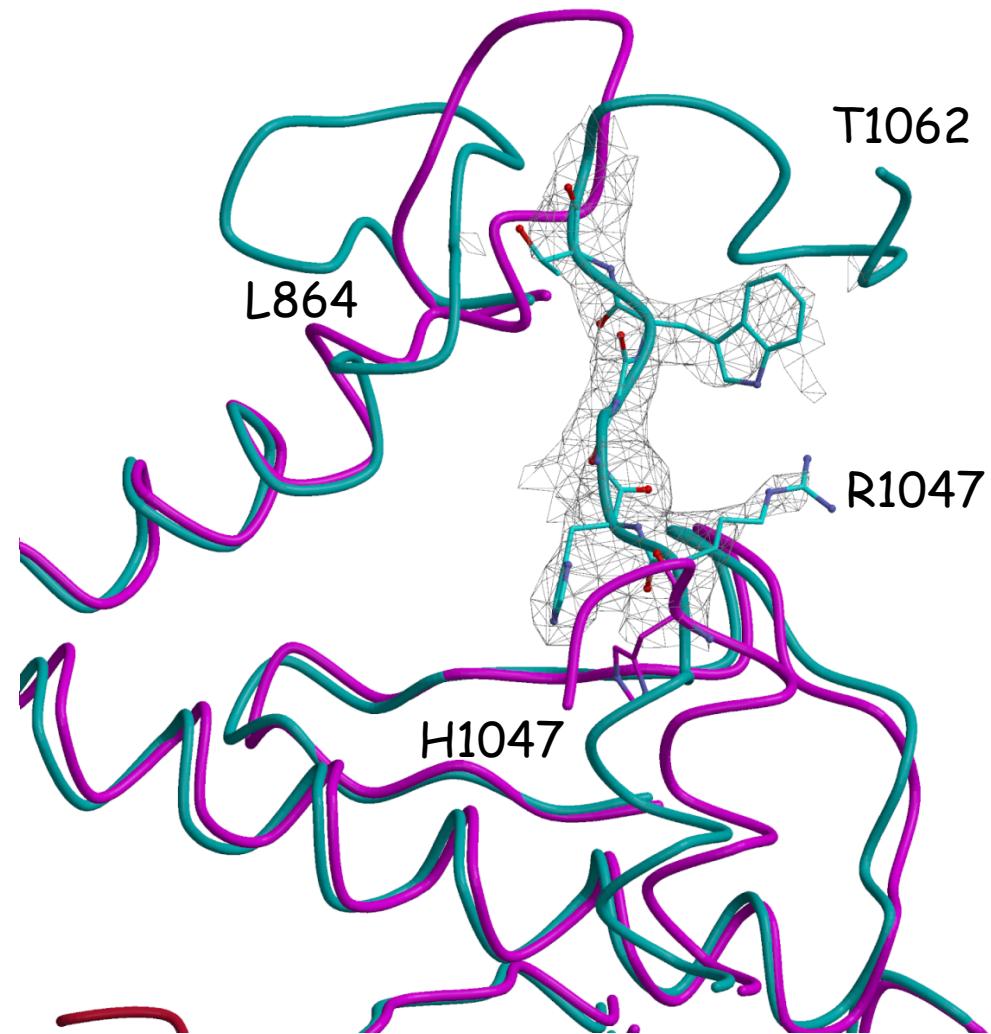
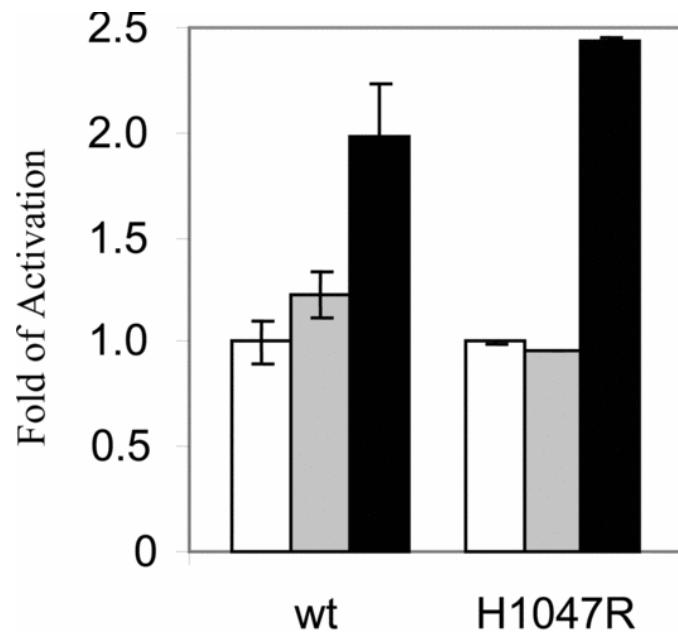
Cancer Specific Mutations: Kinase Domain



Mandelker, et al PNAS (2009) 16996-7001

Carson, et al Biochem. J. (2008) 409, 519-524

Cancer Specific Mutations: Kinase Domain

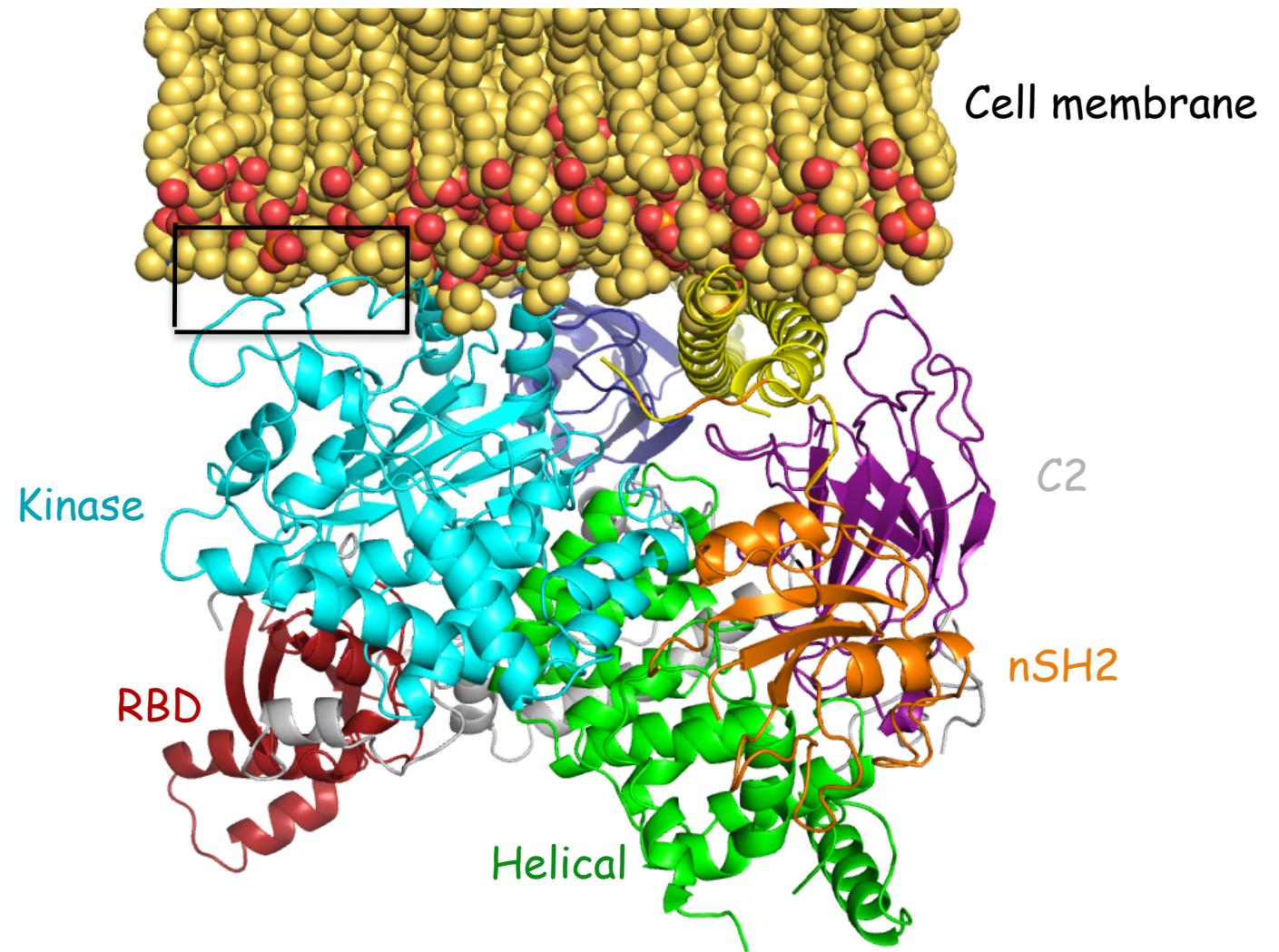


Mandelker, et al PNAS (2009) 16996-7001

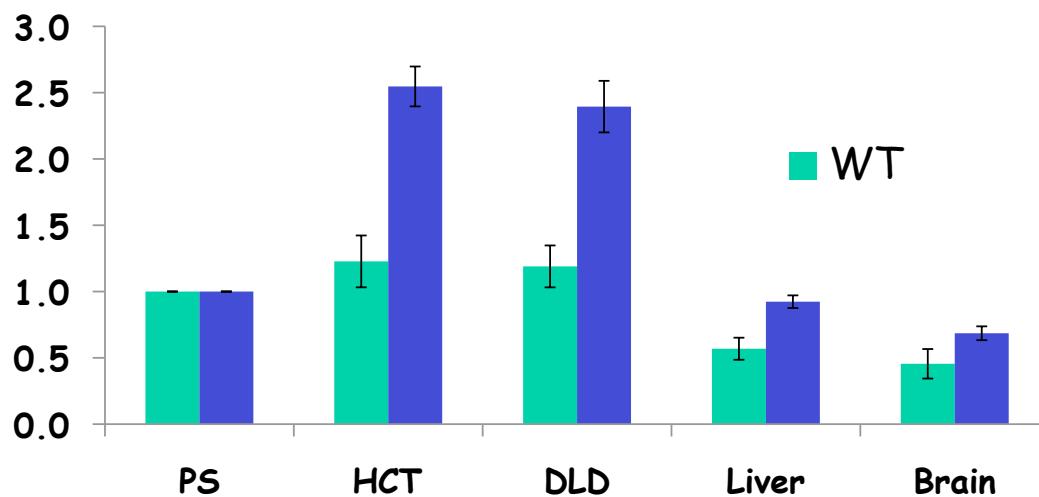
Carson, et al Biochem. J. (2008) 409, 519-524

The H1047R mutation affects the conformation 1047 residue and two loops that contact the cell membrane

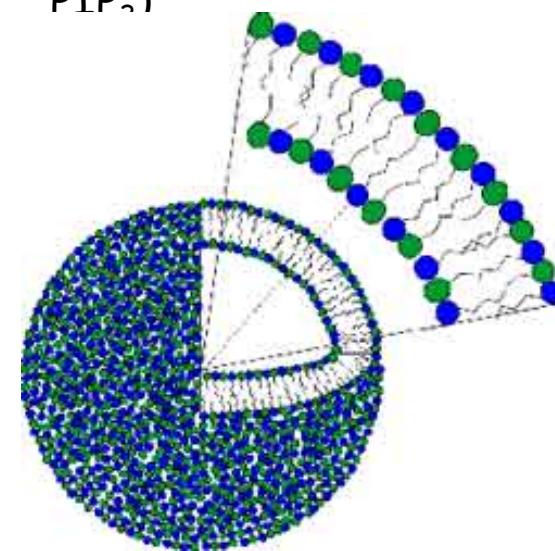
Overall structure of p110 α H1047R/niSH2



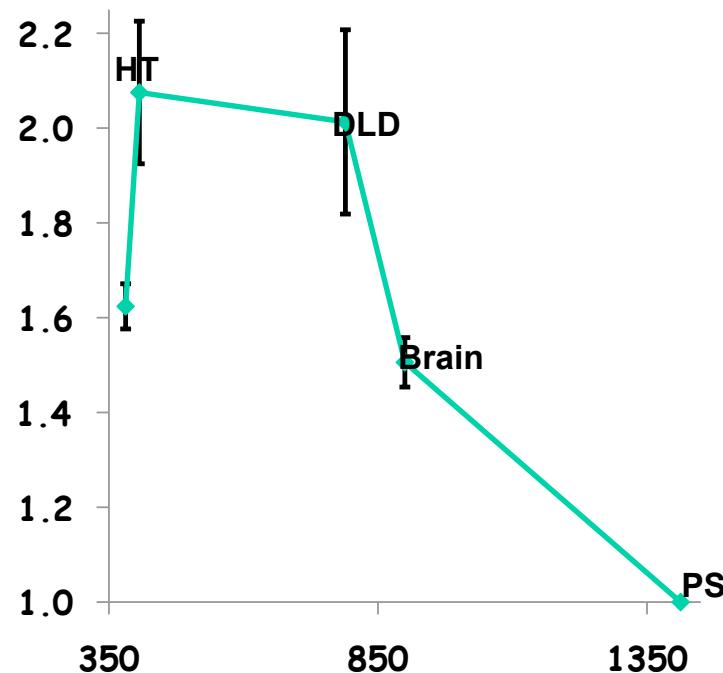
Assay to test effect of lipid membrane composition on PI3Ka enzymatic activity



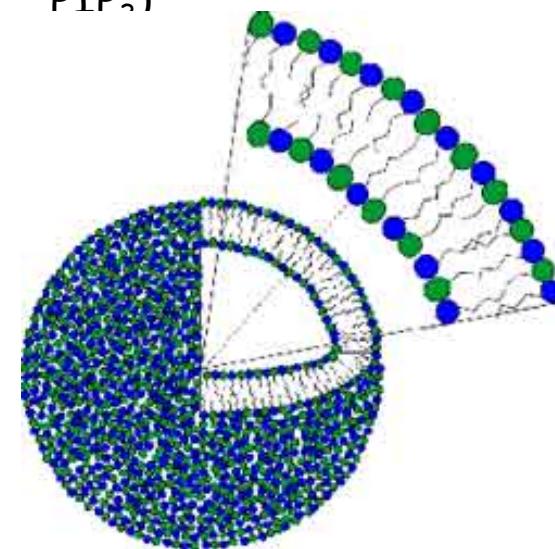
- 1) Generate vesicles of varying lipid compositions.
- 2) Add WT or mutant PI3Ka, PIP₂ substrate, γ -32P-ATP, and MgCl₂
- 3) At varying time points, measure lipid kinase activity (reaction is PIP₂ PIP₁)



H1047R and WT PI3K α are differentially regulated by lipid membrane composition



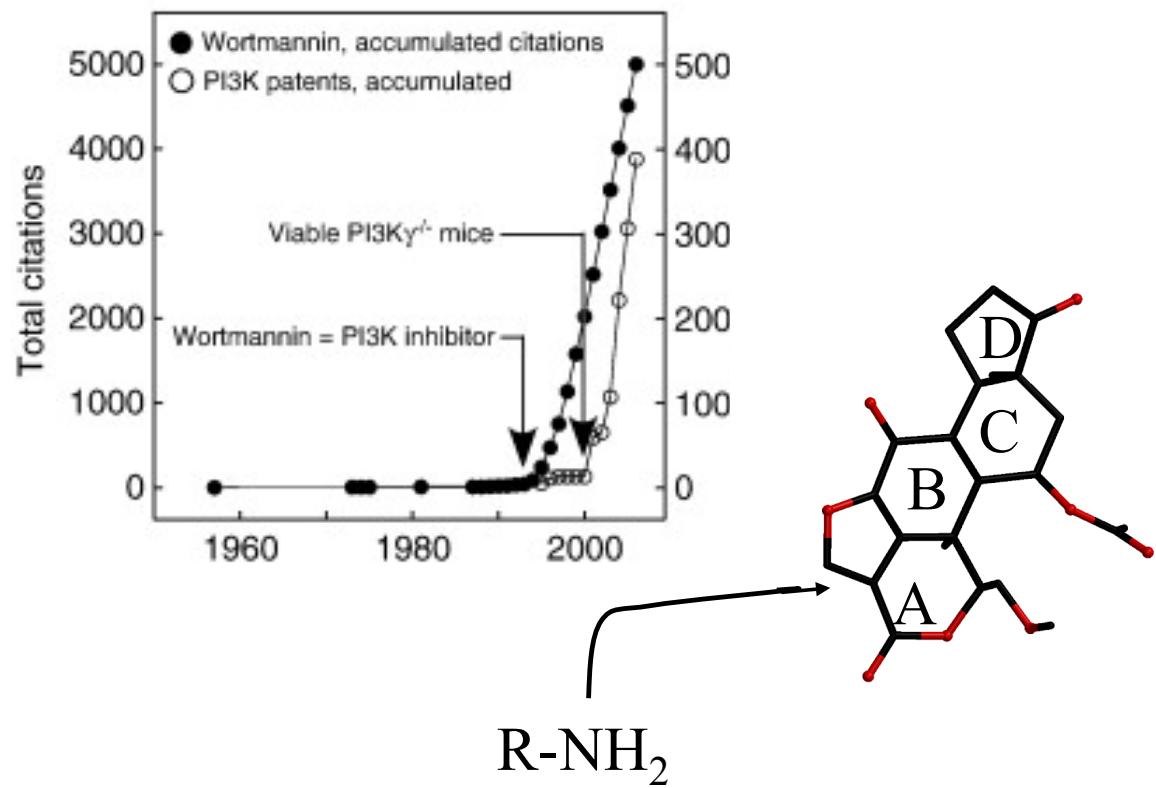
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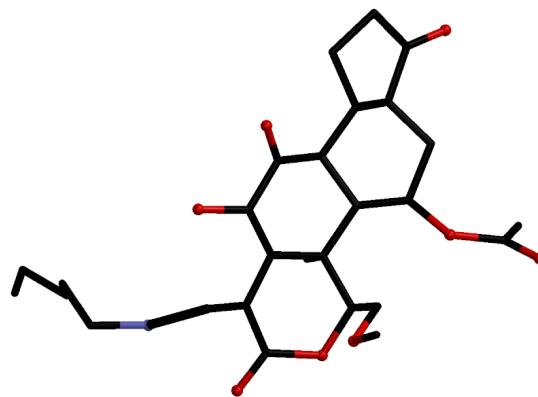
Drug discovery Effort

Wortmannin: PI3K-like inhibitor

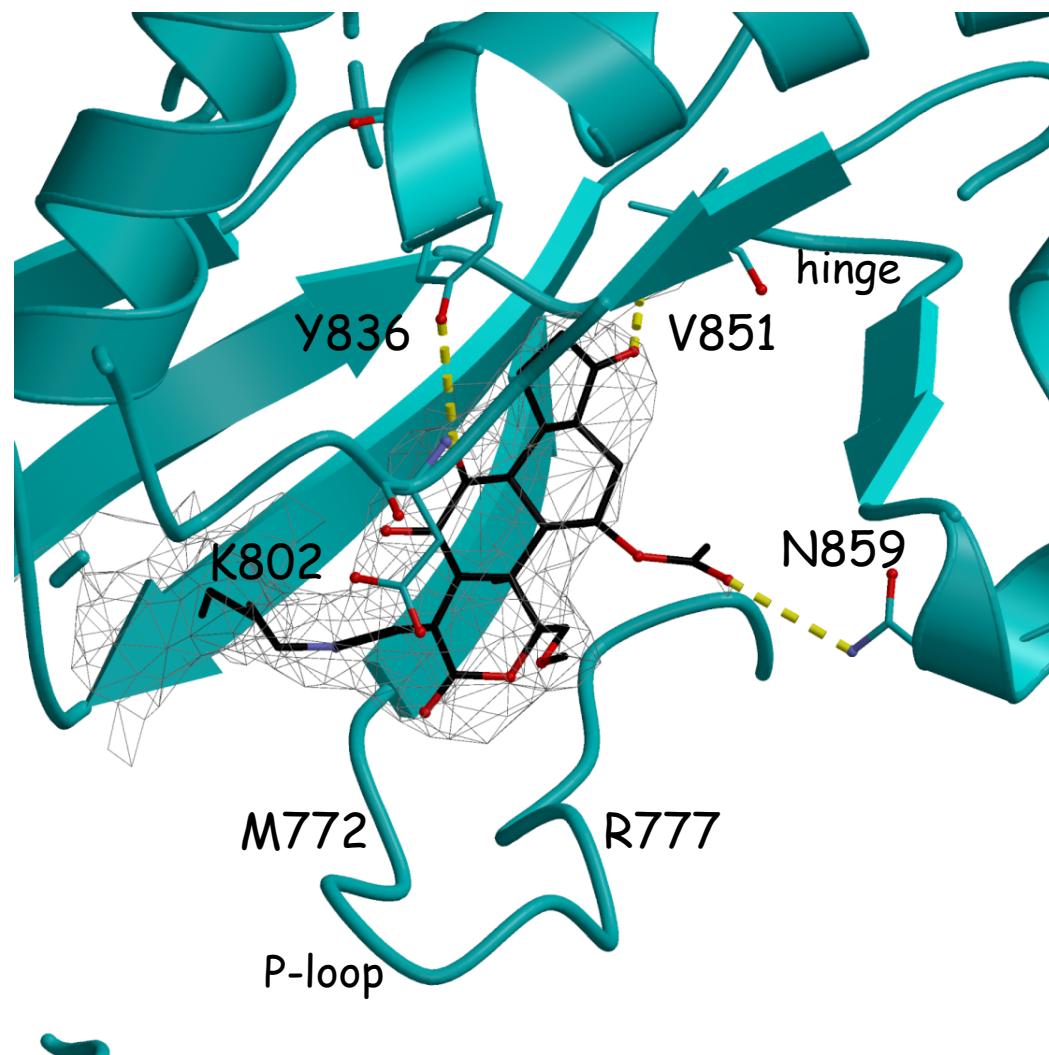
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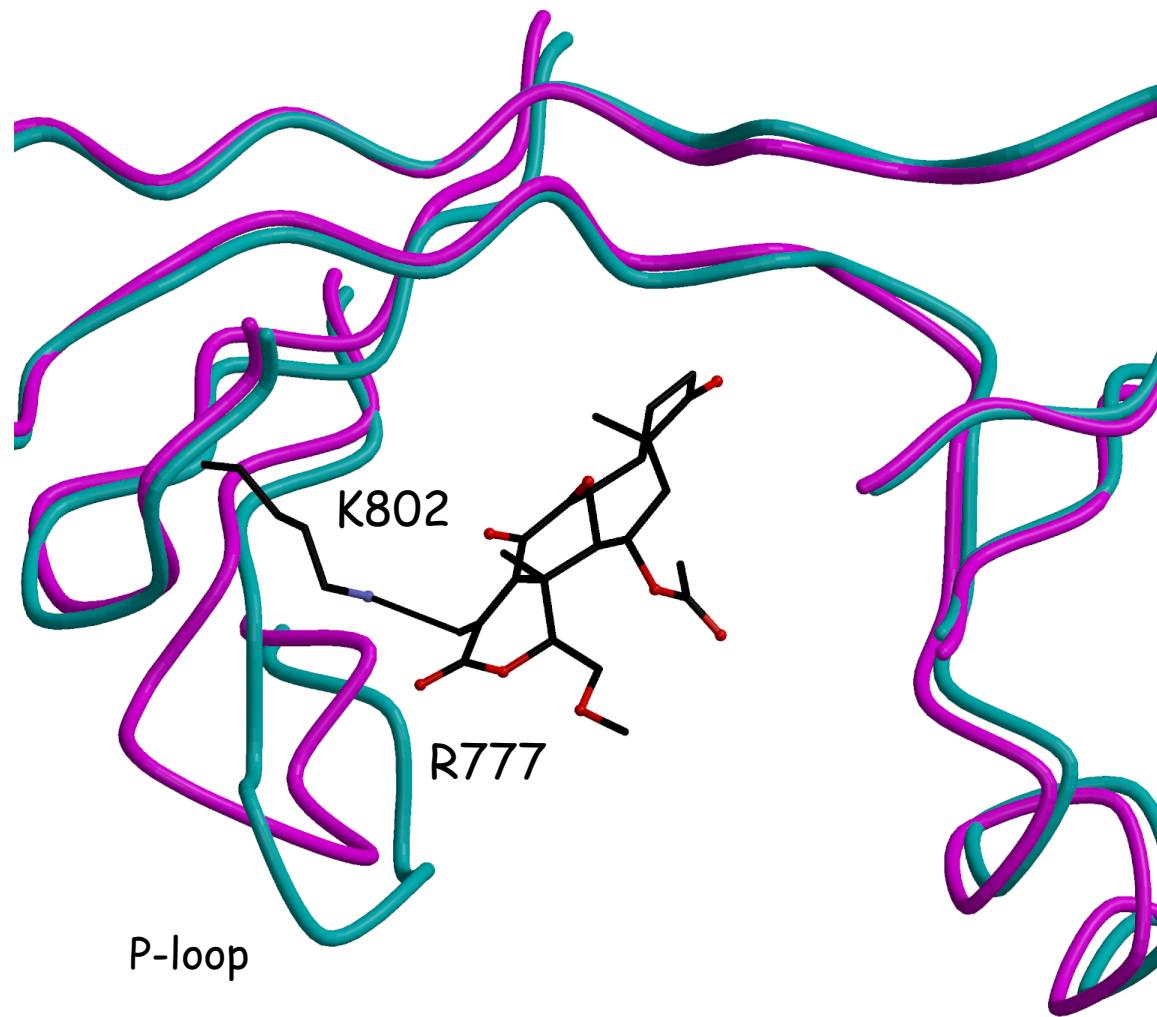
Wortmannin forms 4 H-Bonds and covalently binds Lys802 in ATP binding site



Wortmannin forms 4 H-Bonds and covalently binds Lys802 in ATP binding site

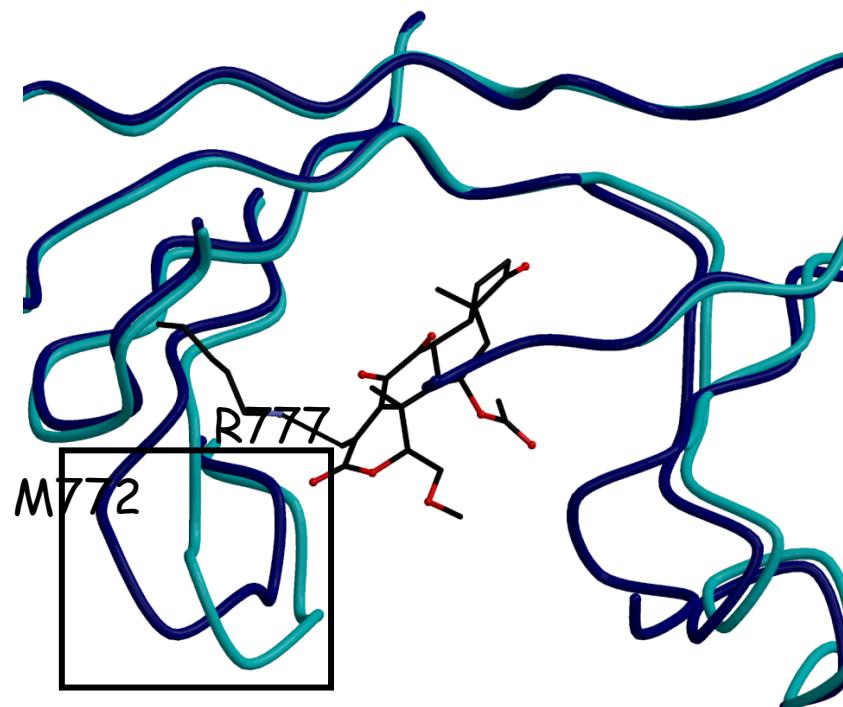


Loop 772-777 in kinase domain shifts conformation upon wortmannin binding

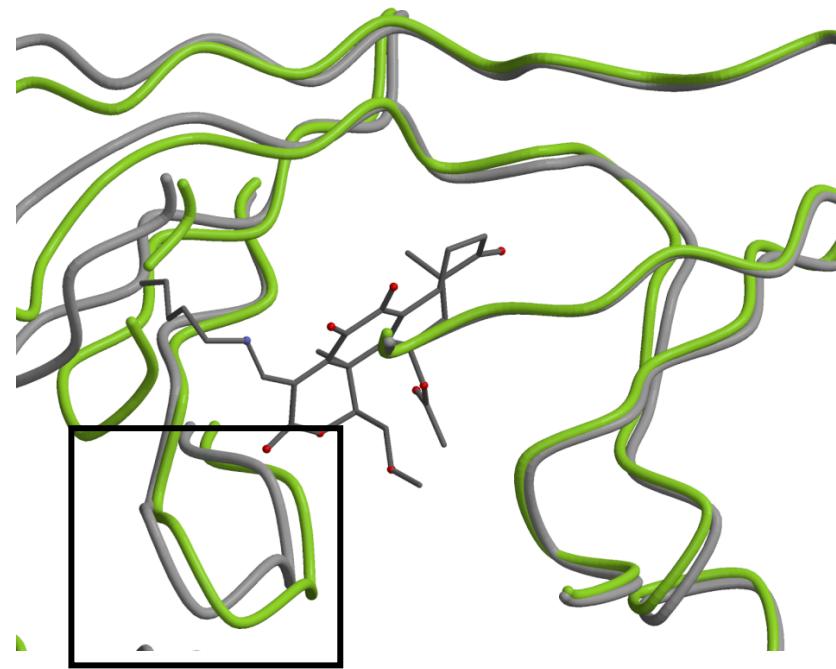


ATP binding site comparison of p110 α and p110 γ shows unique loop shifts upon inhibitor binding

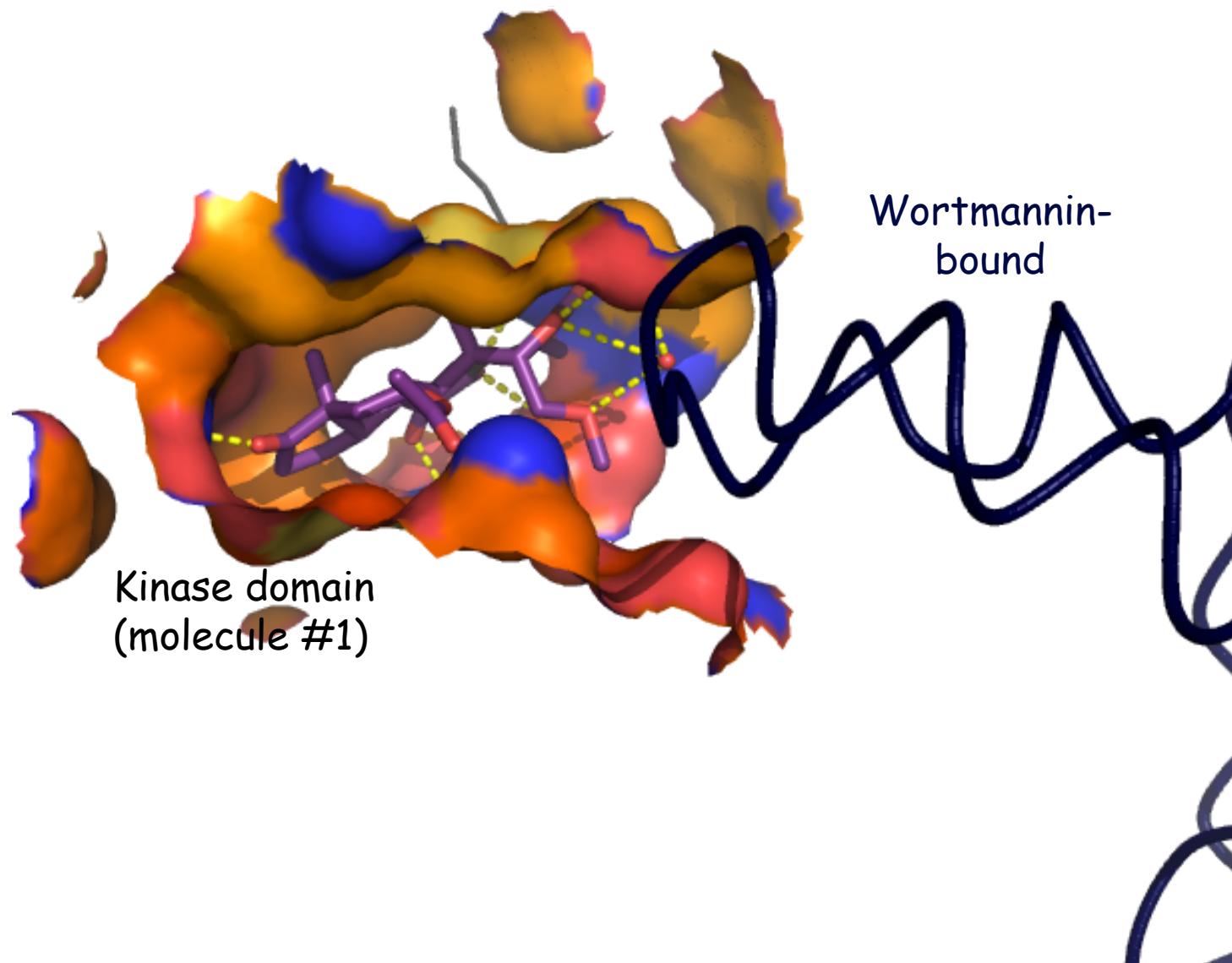
p110 α H1047R free vs. wortmannin-bound



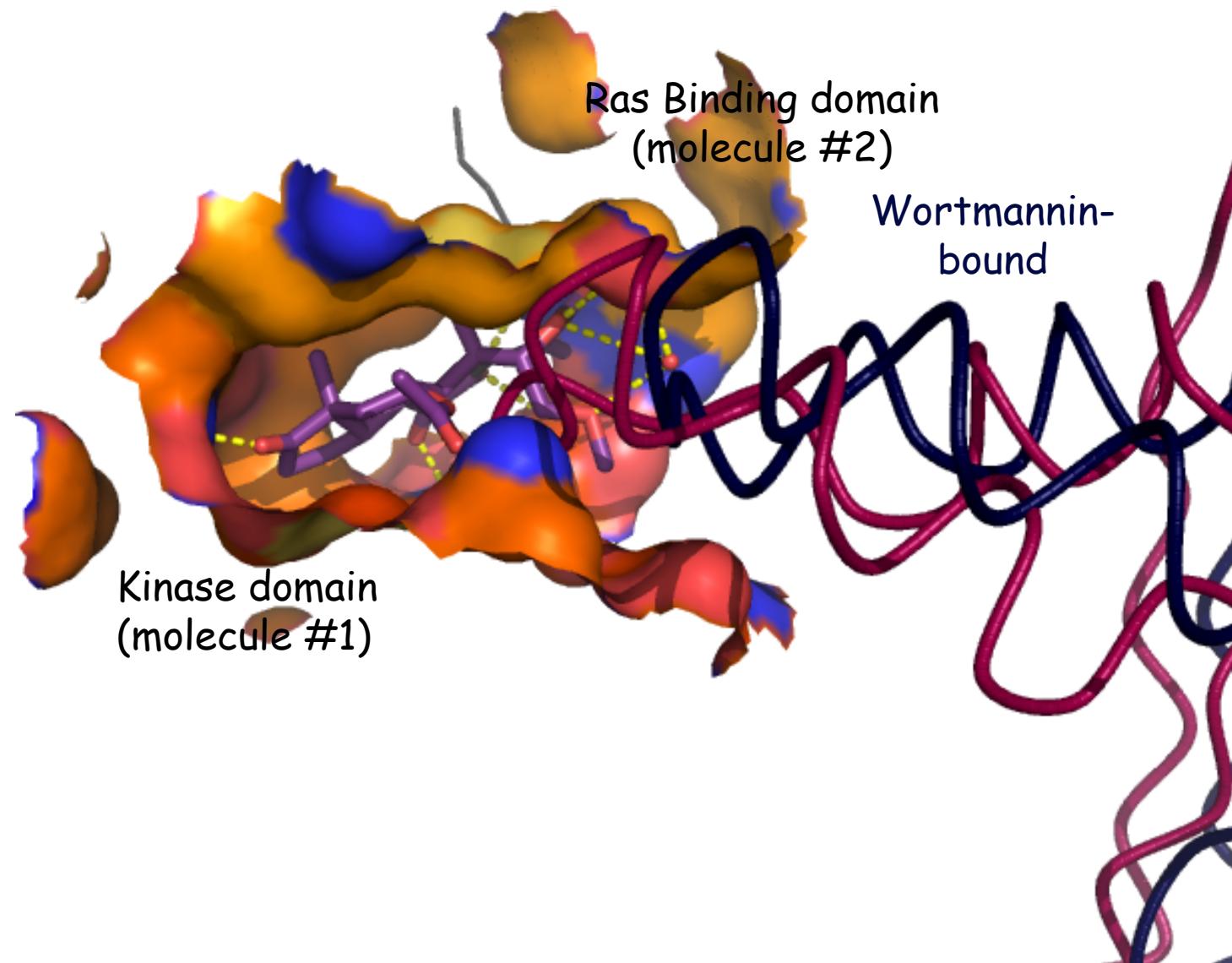
p110 γ free vs. wortmannin-bound



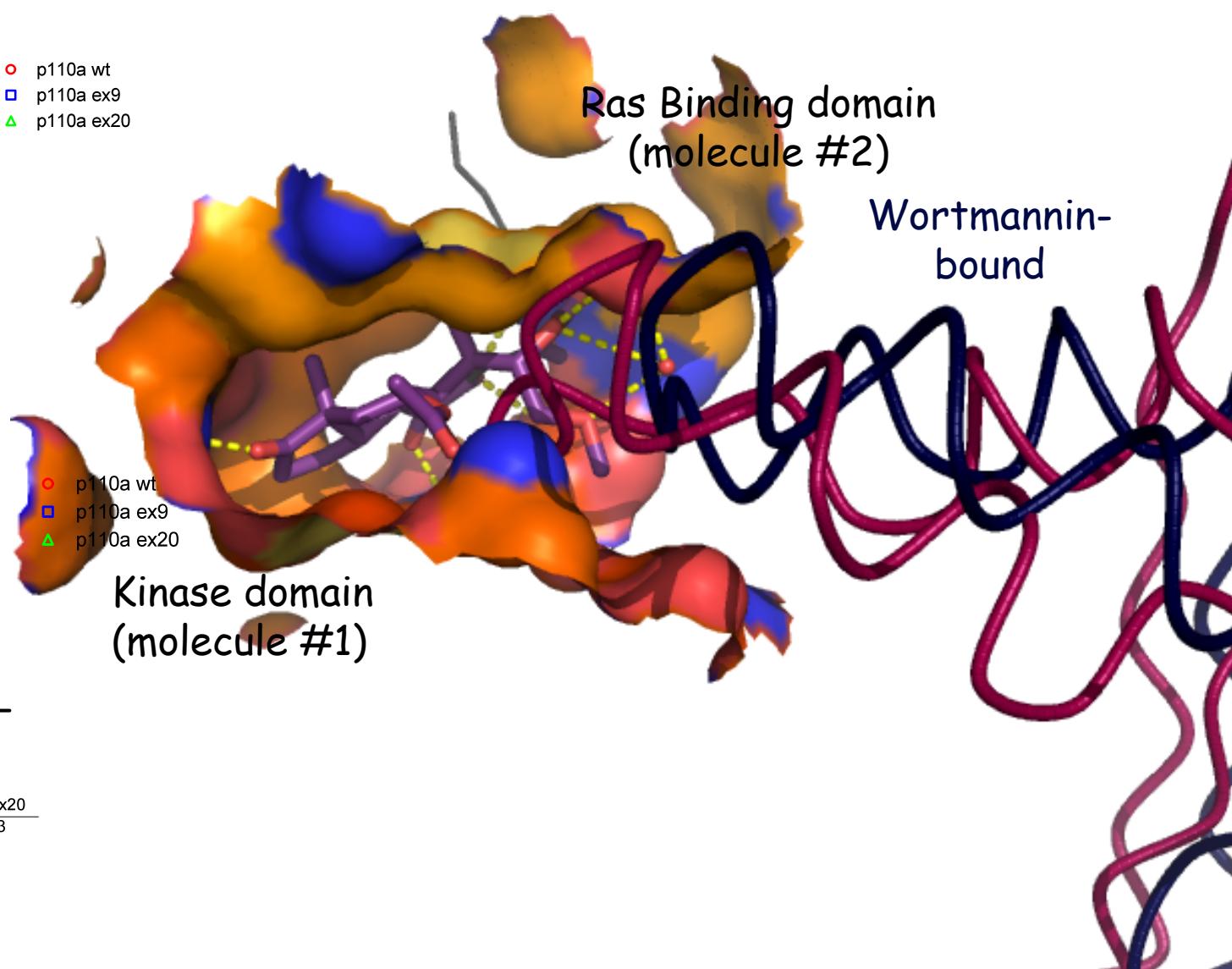
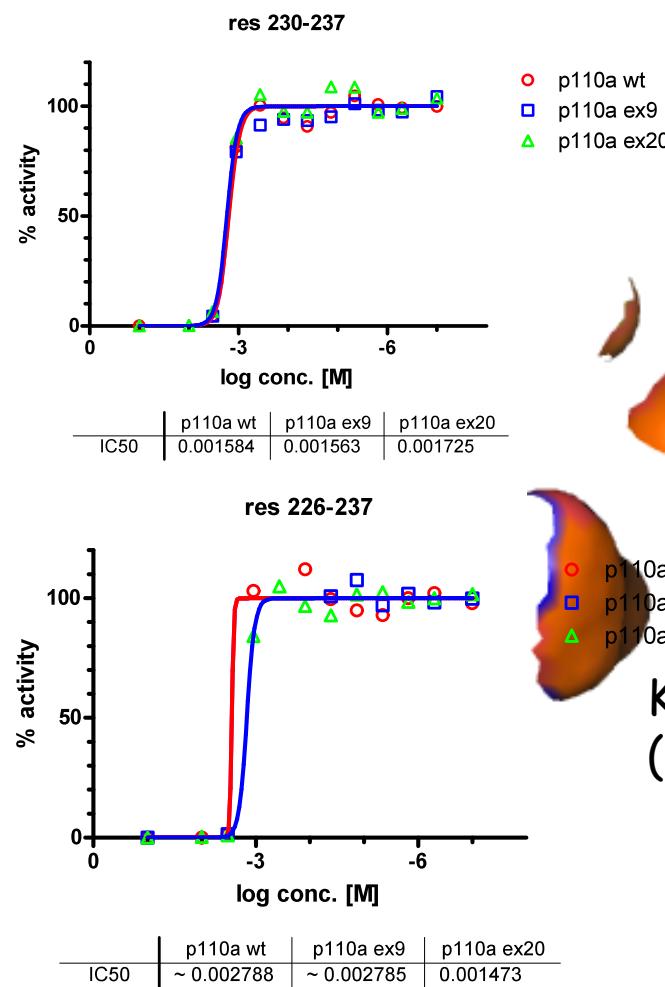
Wortmannin binding causes displacement of symmetry-related Ras Binding Domain



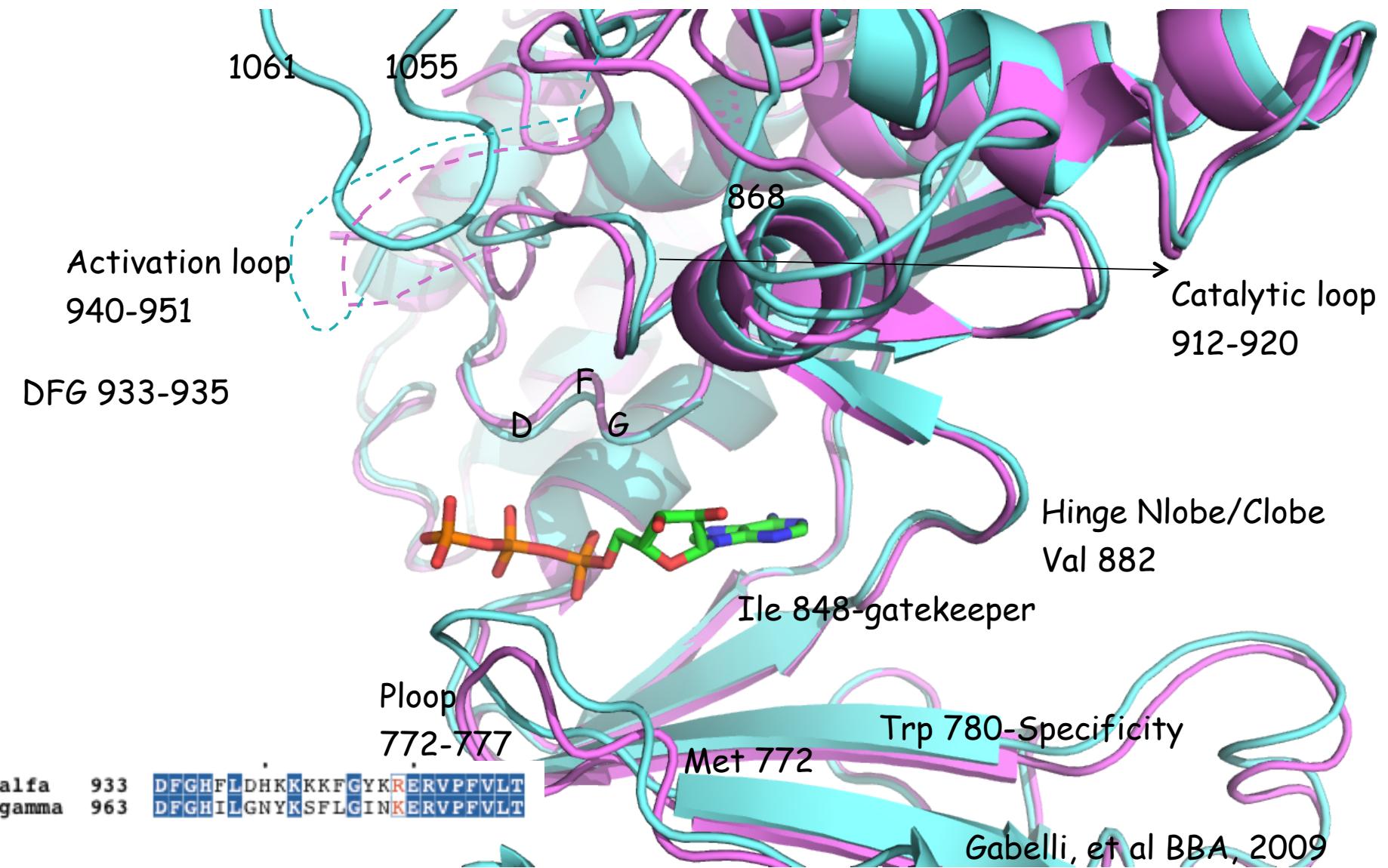
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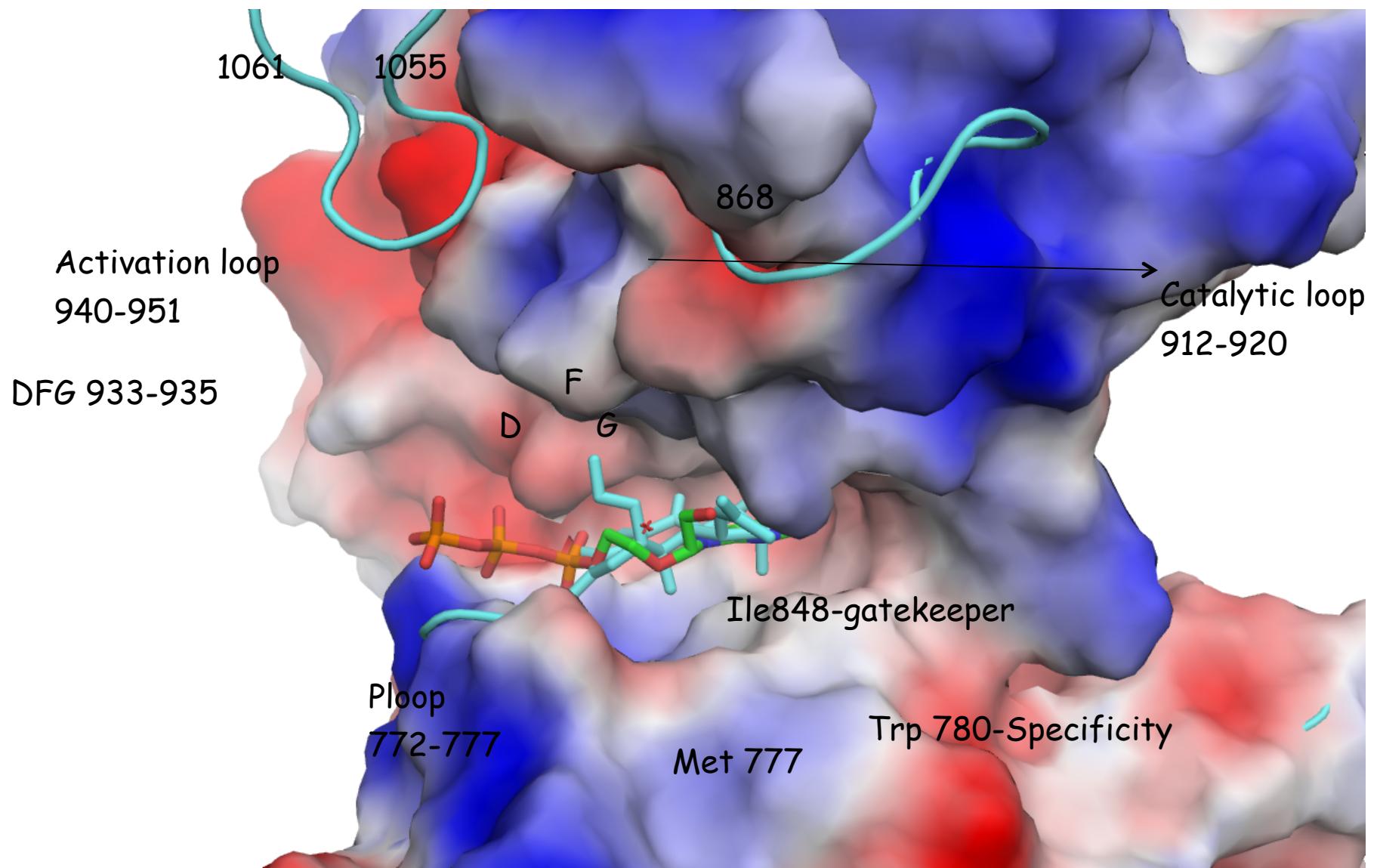
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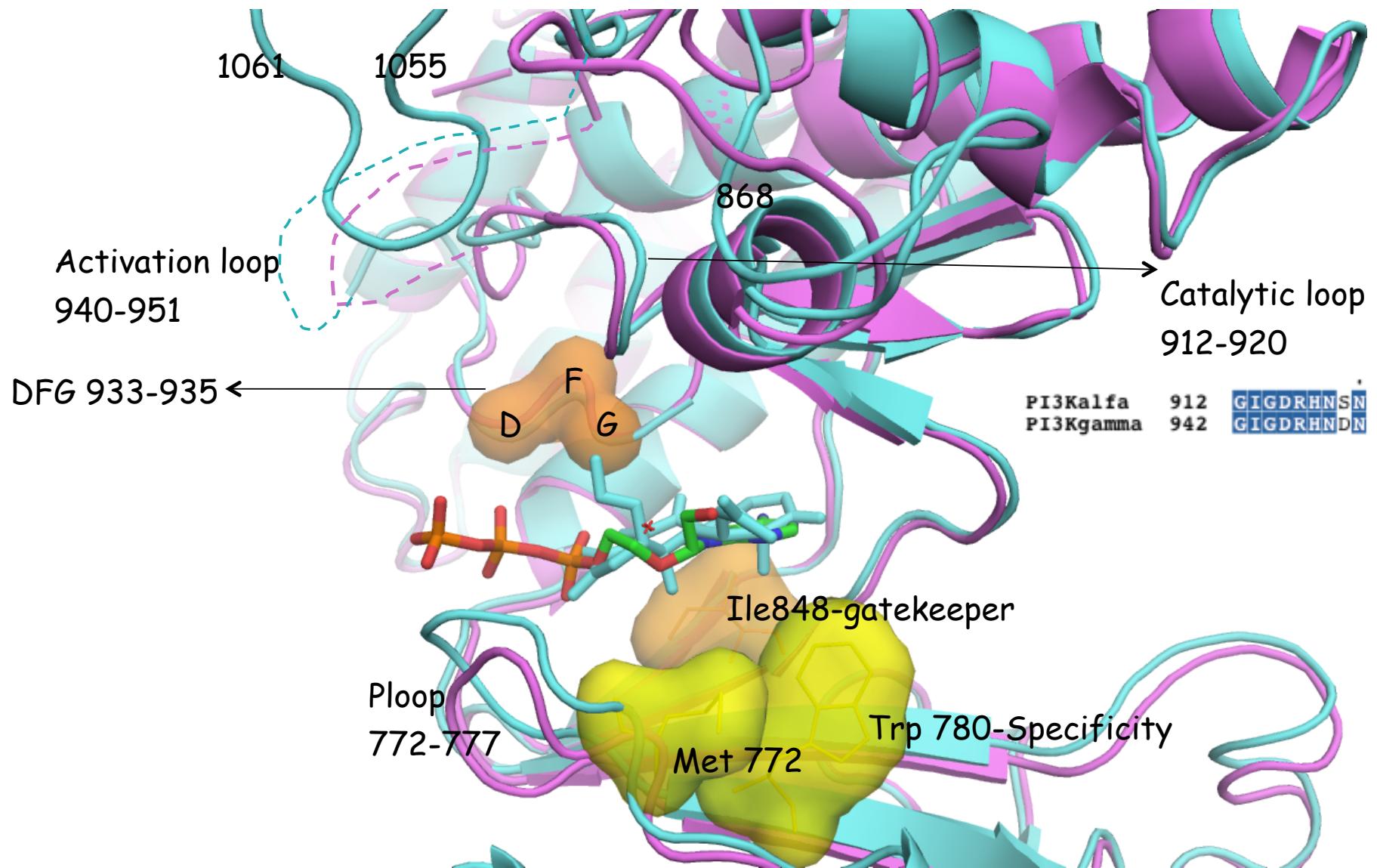
PI3Ka binding site



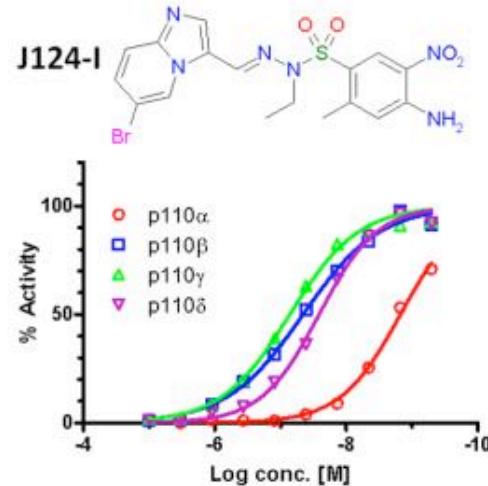
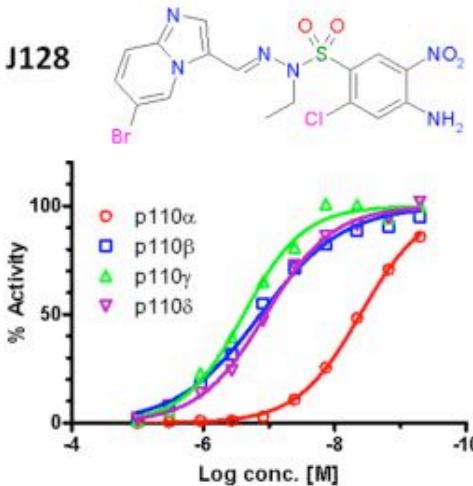
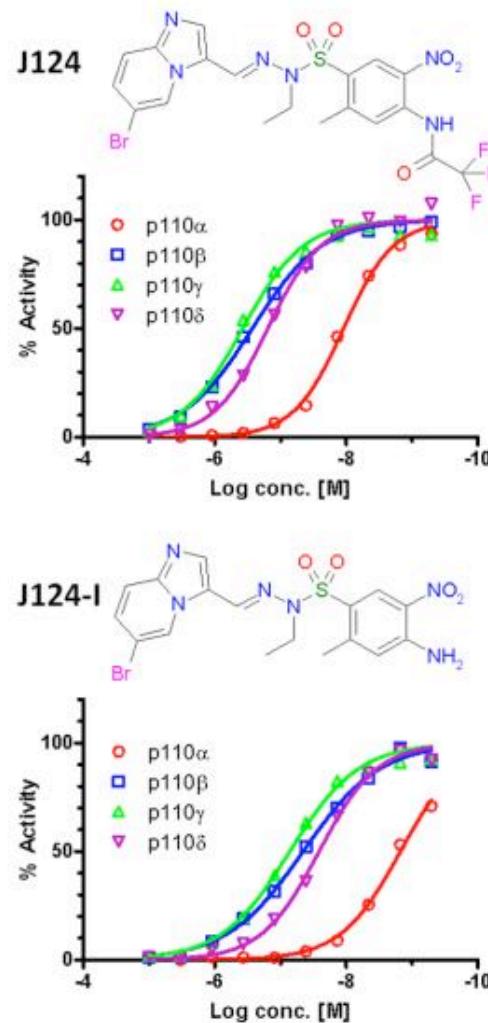
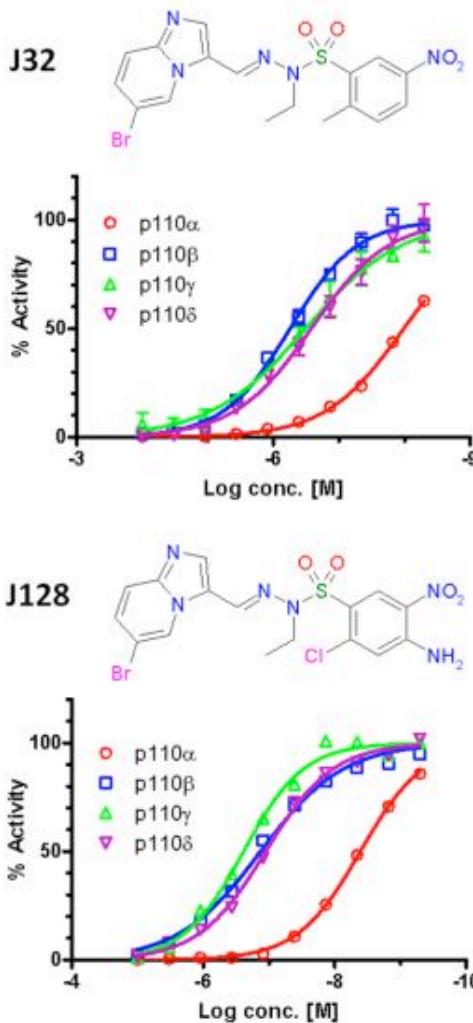
PI3Ka binding site



PI3Ka binding site

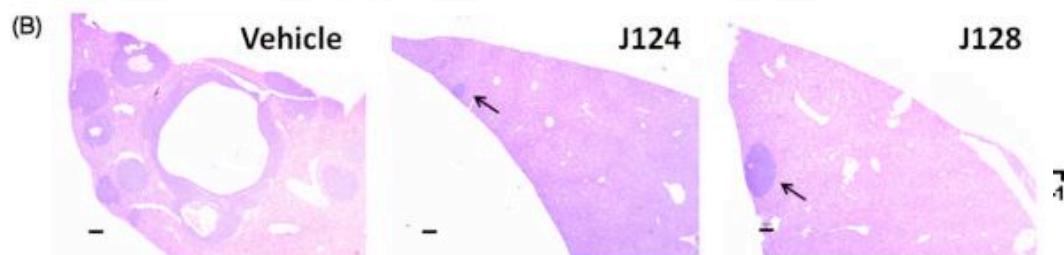
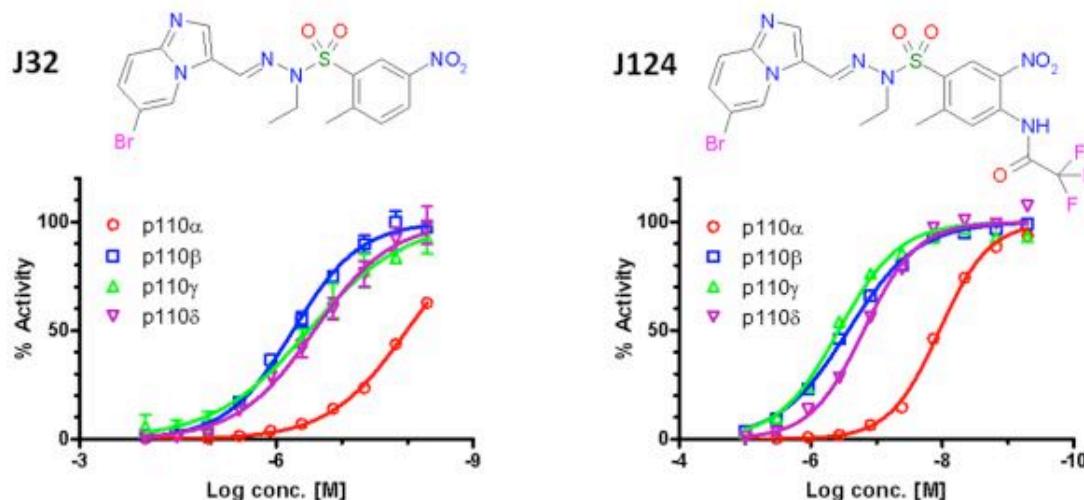


Imidazopyridine based inhibitors: J32 inhibits the AKT pathway



Mandelker, et. al., PNAS., 2009
Schmidt-Kittler, et al., Oncotarget, 2010

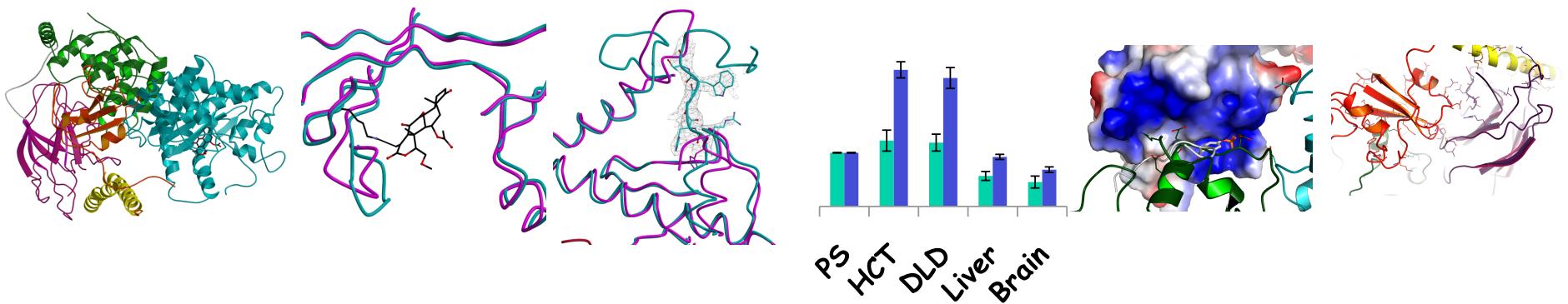
Imidazopyridine based inhibitors: J32 inhibits the AKT pathway



Mandelker, et. al., PNAS., 2009
Schmidt-Kittler, et al., Oncotarget, 2010

Conclusions

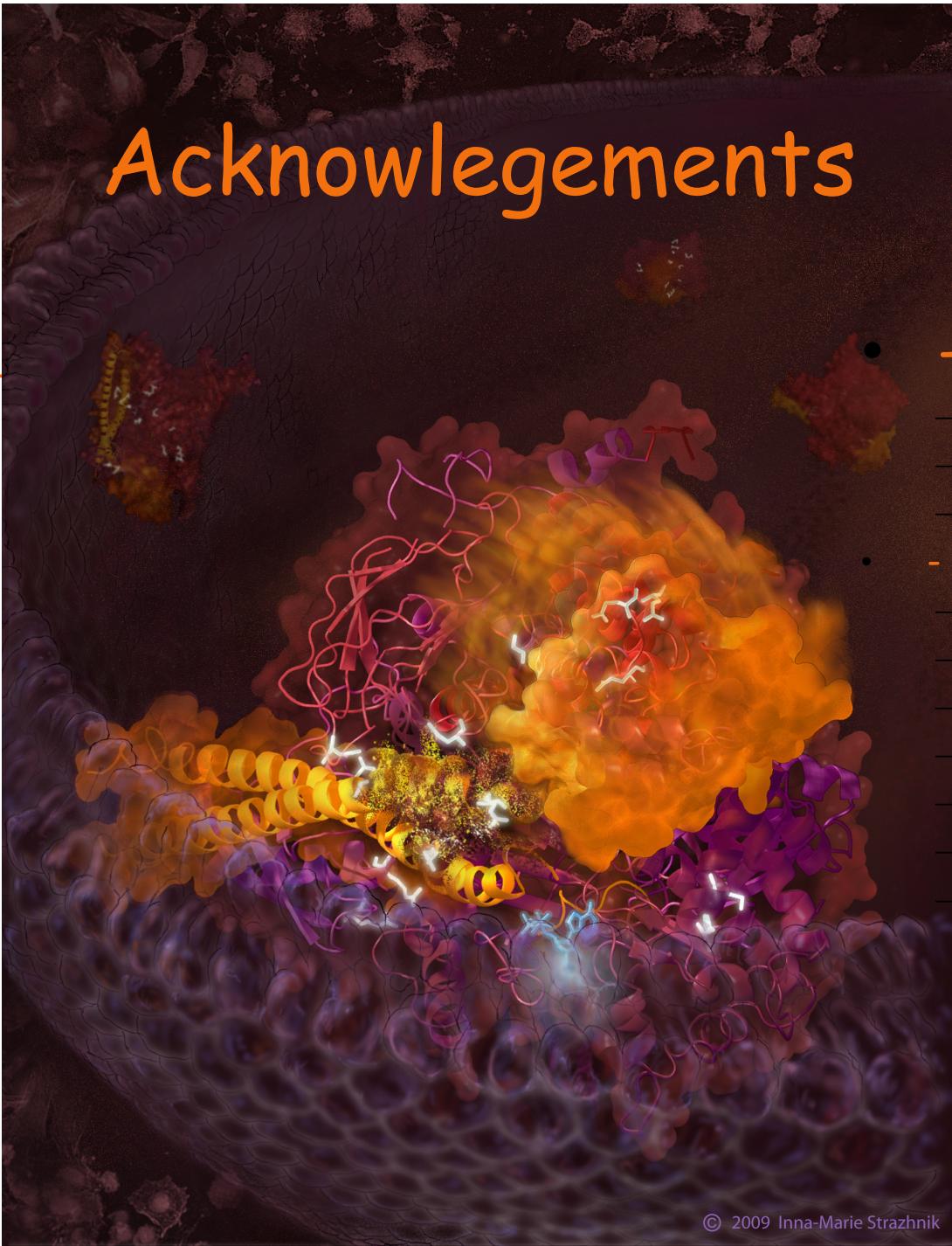
- In the PI3Ka heterodimer, the p85 α nSH2 domain acts as a scaffold, contacting, and possibly coordinating communication between 3 domains of p110 α
- PI3Ka in complex with an inhibitor reveals a loop shift in the ATP binding site, unique to the α -isoform of PI3K.
- The H1047R mutation results in conformational changes in the 1047 residue and in 2 loops that contact the cell membrane.
- The relative lipid kinase activities of the WT and mutant PI3Ka are differentially regulated by lipid membrane composition
- Phospho-peptide binds between nSH2 and helical domain, displacing nSH2
- Movement of nSH2 may open other membrane attachment sites



Acknowledgements

Brookhaven National Lab
X6a, X25, X29

NIH



- Mario Amzel
- Chuan-Hsiang Huang
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- Oleg Schmidt-Kittler
- Yardena Samuels
- Victor Velculescu
- Kenneth W. Kinsler
- Jiuxiang Zhu
- Ian Cheong